

updated  
Search  
L/Gook 4/26/04

incorporation; UGA codon position  
IT Methods & Equipment  
mutagenesis: analytical method, molecular genetics/genetic engineering;  
protein analysis: Analysis/Characterization Techniques: CB, analytical  
method  
ORGN Classifier  
Cercopithecidae 86205  
Super Taxa  
Primates; Mammalia; Vertebrata; Chordata; Animalia  
Organism Name  
COS-7: African green monkey kidney  
Taxa Notes  
Animals, Chordates, Mammals, Nonhuman Mammals, Nonhuman Vertebrates,  
Nonhuman Primates, Primates, Vertebrates  
RN 3614-08-2 (selenocysteine)  
70-18-8 (GLUTATHIONE)

=> d his

(FILE 'HOME' ENTERED AT 15:24:06 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT  
15:24:34 ON 26 APR 2004

L1 19 S GFP AND 157  
L2 24 S GFP AND 172  
L3 4 S L1 AND L2  
L4 1 DUPLICATE REMOVE L3 (3 DUPLICATES REMOVED)  
L5 6 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)  
L6 11 DUPLICATE REMOVE L2 (13 DUPLICATES REMOVED)

=>

d his

(FILE 'HOME' ENTERED AT 14:21:27 ON 26 APR 2004)

FILE 'STNGUIDE' ENTERED AT 14:21:30 ON 26 APR 2004

FILE 'HOME' ENTERED AT 14:21:34 ON 26 APR 2004

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT  
14:22:05 ON 26 APR 2004

L1 301 S (WILD TYPE GFP)  
L2 54 S L1 AND SER?  
L3 53 S L2 AND PRO?  
L4 2 S L3 AND 147

=> s l1 and LEPRAS

L5 0 L1 AND LEPRAS

=>

Connection closed by remote host

d his

(FILE 'HOME' ENTERED AT 14:21:27 ON 26 APR 2004)

FILE 'STNGUIDE' ENTERED AT 14:21:30 ON 26 APR 2004

FILE 'HOME' ENTERED AT 14:21:34 ON 26 APR 2004

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT  
14:22:05 ON 26 APR 2004

L1 301 S (WILD TYPE GFP)  
L2 54 S L1 AND SER?  
L3 53 S L2 AND PRO?  
L4 2 S L3 AND 147

=> s l1 and LEPRAS

L5 0 L1 AND LEPRAS

=>

Connection closed by remote host

d 14 1 all

L4 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
DUPLICATE 1  
AN 2001:538663 BIOSIS  
DN PREV200100538663  
TI Green fluorescent protein rendered susceptible to proteolysis: Positions  
for protease-sensitive insertions.  
AU Chiang, Cheng-Feng; Okou, David T.; Griffin, Tony B.; Verret, C. Reynold;  
Williams, Myron N. V. [Reprint author]  
CS Department of Chemistry, Clark Atlanta University, Atlanta, GA, 30314, USA  
mnwill@cau.edu  
SO Archives of Biochemistry and Biophysics, (October 15, 2001) Vol. 394, No.  
2, pp. 229-235. print.  
CODEN: ABBIA4. ISSN: 0003-9861.  
DT Article  
LA English  
ED Entered STN: 21 Nov 2001  
Last Updated on STN: 25 Feb 2002  
AB The green fluorescent protein (**GFP**) is highly resistant to  
proteolysis and remains uncleaved after prolonged incubation with trypsin  
or pronase despite several putative tryptic and chymotryptic sites in  
exposed loops. We have rendered **GFP** sensitive to proteolysis by  
inserting five amino acids, IEGRS, in loops at position **157**,  
**172**, or 189. Excitation and emission maxima of the three  
insertion mutants were similar to those of wild type, but quantum yields  
of mutants OMEGA172 and OMEGA189 were lower, indicating increased freedom  
of the fluorophore. Trypsin cleaved the native (folded) form of each  
mutant at a unique site defined by the insert. Pronase also yields  
similar digestion patterns in these variants, but further proteolysis was  
also observed, suggesting that the primary cleavage relaxes **GFP**  
structure and reveals previously inaccessible sites. Fluorescence of  
OMEGA189 changed little upon digestion with trypsin but decreased  
progressively by as much as 40% upon digestion with increasing amounts of  
pronase. Fluorescence of other variants was not affected significantly by  
the proteases, further confirming the remarkable stabilities of  
**GFP** variants. These constructs define a new conformation-  
sensitive site around residue 189 of **GFP** and show that  
**GFP** may be useful for design of protease-susceptible molecules for  
monitoring of specific proteolytic activities in vivo.  
CC Biochemistry studies - General 10060  
Biochemistry studies - Proteins, peptides and amino acids 10064  
Enzymes - General and comparative studies: coenzymes 10802  
Physiology and biochemistry of bacteria 31000  
IT Major Concepts  
Biochemistry and Molecular Biophysics  
IT Chemicals & Biochemicals  
factor Xa; green fluorescent protein; pronase; protease; trypsin  
IT Methods & Equipment  
mutagenesis: analytical method, genetic engineering, genetic method  
IT Miscellaneous Descriptors  
protease-sensitive insertions; proteolysis  
ORGN Classifier  
Bacteria 05000  
Super Taxa  
Microorganisms  
Organism Name  
bacteria  
Taxa Notes  
Bacteria, Eubacteria, Microorganisms  
RN 9002-05-5 (factor Xa)  
9036-06-0 (pronase)

9001-92-7 (protease)  
9002-07-7 (trypsin)

=> d his

(FILE 'HOME' ENTERED AT 15:24:06 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT  
15:24:34 ON 26 APR 2004

L1 19 S GFP AND 157  
L2 24 S GFP AND 172  
L3 4 S L1 AND L2  
L4 1 DUPLICATE REMOVE L3 (3 DUPLICATES REMOVED)

=> duplicate remove l1

DUPLICATE PREFERENCE IS 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT'  
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n  
PROCESSING COMPLETED FOR L1  
L5 6 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)

=> d l5 1-6 all

L5 ANSWER 1 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
DUPLICATE 1  
AN 2003:261418 BIOSIS  
DN PREV200300261418  
TI The sigmaE regulon and the identification of additional sporulation genes  
in Bacillus subtilis.  
AU Eichenberger, Patrick; Jensen, Shane T.; Conlon, Erin M.; van Ooij,  
Christiaan; Silvaggi, Jessica; Gonzalez-Pastor, Jose-Eduardo; Fujita,  
Masaya; Ben-Yehuda, Sigal; Stragier, Patrick; Liu, Jun S.; Losick, Richard  
[Reprint Author]  
CS Department of Molecular and Cellular Biology, Harvard University  
Biological Laboratories, 16 Divinity Avenue, Cambridge, MA, 02138, USA  
losick@mcb.harvard.edu  
SO Journal of Molecular Biology, (11 April 2003) Vol. 327, No. 5, pp.  
945-972. print.  
ISSN: 0022-2836 (ISSN print).  
DT Article  
LA English  
ED Entered STN: 4 Jun 2003  
Last Updated on STN: 4 Jun 2003  
AB We report the identification and characterization on a genome-wide basis  
of genes under the control of the developmental transcription factor  
sigmaE in Bacillus subtilis. The sigmaE factor governs gene expression in  
the larger of the two cellular compartments (the mother cell) created by  
polar division during the developmental process of sporulation. Using  
transcriptional profiling and bioinformatics we show that 253 genes  
(organized in 157 operons) appear to be controlled by sigmaE.  
Among these, 181 genes (organized in 121 operons) had not been previously  
described as members of this regulon. Promoters for many of the newly  
identified genes were located by transcription start site mapping. To  
assess the role of these genes in sporulation, we created null mutations  
in 98 of the newly identified genes and operons. Of the resulting  
mutants, 12 (in prkA, ybaN, ybhH, ykvV, ylbJ, ypbJ, yqfC, yqfD, ytrH,  
ytrI, ytvI and yunB) exhibited defects in spore formation. In addition,  
subcellular localization studies were carried out using in-frame fusions  
of several of the genes to the coding sequence for GFP. A  
majority of the fusion proteins localized either to the membrane  
surrounding the developing spore or to specific layers of the spore coat,  
although some fusions showed a uniform distribution in the mother cell

cytoplasm. Finally, we used comparative genomics to determine that 46 of the sigmaE-controlled genes in *B. subtilis* were present in all of the Gram-positive endospore-forming bacteria whose genome has been sequenced, but absent from the genome of the closely related but not endospore-forming bacterium *Listeria monocytogenes*, thereby defining a core of conserved sporulation genes of probable common ancestral origin. Our findings set the stage for a comprehensive understanding of the contribution of a cell-specific transcription factor to development and morphogenesis.

CC Cytology - General 02502  
 Genetics - General 03502  
 Biochemistry studies - Nucleic acids, purines and pyrimidines 10062  
 Development and Embryology - General and descriptive 25502  
 Morphology and cytology of bacteria 30500  
 Physiology and biochemistry of bacteria 31000  
 Genetics of bacteria and viruses 31500

IT Major Concepts  
 Cell Biology; Development; Molecular Genetics (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals  
 sigma factors; sigma-E: developmental transcription factor; sporulation genes: identification

IT Methods & Equipment  
 cytology: histology and cytology techniques, laboratory techniques

IT Miscellaneous Descriptors  
 bacterial genomics; bioinformatics; genome; polar division; protein localization; sigma-E regulation; sporulation

ORGN Classifier  
 Endospore-forming Gram-Positives 07810  
 Super Taxa  
 Eubacteria; Bacteria; Microorganisms  
 Organism Name  
 Bacillus subtilis (species): sporulation  
 Taxa Notes  
 Bacteria, Eubacteria, Microorganisms

ORGN Classifier  
 Regular Nonsporing Gram-Positive Rods 07830  
 Super Taxa  
 Eubacteria; Bacteria; Microorganisms  
 Organism Name  
 Listeria monocytogenes (species)  
 Taxa Notes  
 Bacteria, Eubacteria, Microorganisms

L5 ANSWER 2 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
 AN 2004:180837 BIOSIS  
 DN PREV200400180886  
 TI Hereditary spherocytosis mutations in an erythroid ankyrin insulator element are associated with gene silencing in vitro and promoter dysfunction in transgenic mice.  
 AU Liem, Robert I. [Reprint Author]; Seidel, Nancy E. [Reprint Author]; Wong, Clara; Cline, Amanda P. [Reprint Author]; Gallagher, Patrick G.; Bodine, David M. [Reprint Author]  
 CS Hematopoiesis Section, NHGRI, Bethesda, MD, USA  
 SO Blood, (November 16 2003) Vol. 102, No. 11, pp. 7a. print.  
 Meeting Info.: 45th Annual Meeting of the American Society of Hematology. San Diego, CA, USA. December 06-09, 2003. American Society of Hematology. CODEN: BLOOAW. ISSN: 0006-4971.  
 DT Conference; (Meeting)  
 Conference; Abstract; (Meeting Abstract)  
 LA English  
 ED Entered STN: 7 Apr 2004

Last Updated on STN: 7 Apr 2004

- AB Mutations at positions -108 and -153 of the human erythroid ankyrin-1 (ANK-1) promoter are associated with non-dominant hereditary spherocytosis, resulting in hemolytic anemia. However, the mechanism by which the mutations cause ankyrin deficiency is unknown. Using a transgenic mouse assay, we have shown that the ANK-1 promoter containing the -108/-153 mutations fused to the human gamma-globin gene resulted in low-level, position dependent, copy number independent and variegated expression of the transgene (17 lines) when compared to 32 lines containing the wild type (WT) promoter. Normal function of the -108/-153 promoter was restored by flanking the transgene with the insulator from the chicken beta-globin locus (5'HS4-chHS4). Insulators are associated with DNase I hypersensitive sites (HS) at the boundary between chromatin that is resistant to DNase I digestion and DNase I sensitive chromatin. Insulators are defined by a "barrier" function, which prevents gene silencing, and may also have enhancer blocking activity. We hypothesized that the WT ANK-1 promoter contains an insulator that is disrupted by the -108/-153 mutations. To test this hypothesis, we digested nuclei from K562 cells with increasing amounts of DNase I and demonstrated a 200 bp HS over the erythroid ANK-1 promoter between -98 and -296. No HS were detected in a 10 kb region of DNase I resistant chromatin upstream of the ANK-1 promoter, and DNase I sensitive chromatin extended at least 2 kb downstream, demonstrating that the HS is at the boundary between DNase I resistant and sensitive chromatin. Using chromatin immunoprecipitation (ChIP), we demonstrated that GATA-1 and CBP occupied the region between -157 and +69 of the WT ANK-1 promoter and that histones H3 and H4 were hyperacetylated in this region. Gel shift assays with the WT promoter showed that Brg-1, which provides the ATPase activity of the SWI/SNF chromatin remodeling complex and is associated with barrier function in chHS4, and CTCF, which is required for enhancer blocking in chHS4, bound to the sequence between -99 and -159, but not to the same sequence with the -108/-153 mutations. DNase I treatment of fetal liver cells from transgenic mice revealed the ANK-1 HS was present in animals containing the WT promoter, but HS formation was inhibited by the -108/-153 mutations. We tested the sequence between -98 and -296 from both the WT and the -108/-153 ANK-1 promoter for its ability to suppress gene silencing in vitro. FACS analysis of K562 cells transfected with a construct containing HS2 from the human beta-globin locus and the gamma-globin promoter linked to the GFP gene showed rapid silencing of the transgene in 8/8 cell lines. Gene silencing was not observed in cell lines transfected with either 2 (12 cell lines) or 4 copies (12 cell lines) of the WT ANK-1 sequence flanking the transgene. Gene silencing was observed in 5/10 cell lines containing 2 copies of the -108/-153 ANK-1 sequence flanking the transgene ( $\chi^2=14.95$ ,  $p<0.001$ ). Similar to the insulator at the 3' end of the chicken beta-globin locus, the WT ANK-1 sequence did not block the effects of the HS2 enhancer in K562 cells. We conclude that the erythroid ANK-1 promoter contains an insulator that is associated with a DNase I HS and that the -108/-153 mutations disturb binding of critical chromatin remodeling proteins, leading to position effects in transgenic mice and gene silencing in K562 cells. We propose the -108/-153 mutations may lead to ankyrin deficient erythrocytes in spherocytosis patients by a similar mechanism.
- CC General biology - Symposia, transactions and proceedings 00520  
Genetics - General 03502  
Genetics - Animal 03506  
Genetics - Human 03508  
Blood - Blood and lymph studies 15002  
Blood - Blood cell studies 15004  
Blood - Blood, lymphatic and reticuloendothelial pathologies 15006
- IT Major Concepts  
Blood and Lymphatics (Transport and Circulation); Molecular Genetics (Biochemistry and Molecular Biophysics)

IT Parts, Structures, & Systems of Organisms  
 blood: blood and lymphatics; erythroid: blood and lymphatics

IT Diseases  
 hereditary spherocytosis: blood and lymphatic disease, genetic disease, genetics  
 Spherocytosis, Hereditary (MeSH)

IT Methods & Equipment  
 gene silencing: genetic techniques, laboratory techniques

ORGN Classifier  
 Hominidae 86215  
 Super Taxa  
 Primates; Mammalia; Vertebrata; Chordata; Animalia  
 Organism Name  
 K562 cell line (cell line): human chronic myelogenous leukemia cells  
 human (common)  
 Taxa Notes  
 Animals, Chordates, Humans, Mammals, Primates, Vertebrates

ORGN Classifier  
 Muridae 86375  
 Super Taxa  
 Rodentia; Mammalia; Vertebrata; Chordata; Animalia  
 Organism Name  
 mouse (common): transgenic  
 Taxa Notes  
 Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

GEN human ANK-1 gene [human ankyrin-1 gene] (Hominidae): mutation, promoter region; human alpha-globin gene (Hominidae): promoter region; human beta-globin gene (Hominidae): locus

L5 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
 DUPLICATE 2

AN 2002:337041 BIOSIS

DN PREV200200337041

TI Transduction of interphase cells by avian sarcoma virus.

AU Katz, Richard A. [Reprint author]; Greger, James G.; Darby, Kristen; Boimel, Pamela; Rall, Glenn F.; Skalka, Anna Marie

CS Fox Chase Cancer Center, Institute for Cancer Research, 7701 Burholme Ave., Philadelphia, PA, 19111, USA  
 R\_Katz@fccc.edu

SO Journal of Virology, (June, 2002) Vol. 76, No. 11, pp. 5422-5434. print.  
 CODEN: JOVIAM. ISSN: 0022-538X.

DT Article

LA English

ED Entered STN: 12 Jun 2002  
 Last Updated on STN: 12 Jun 2002

AB It has been generally believed that oncoretroviruses are dependent on mitosis for efficient nuclear entry of viral DNA. We previously identified a nuclear localization signal in the integrase protein of an oncoretrovirus, avian sarcoma virus (ASV), suggesting an active import mechanism for the integrase-DNA complex (G. Kukolj, R. A. Katz, and A. M. Skalka, Gene 223:157-163, 1998). Here, we have evaluated the requirement for mitosis in nuclear import and integration of ASV DNA. Using a modified ASV encoding a murine leukemia virus amphotropic env gene and a green fluorescent protein (GFP) reporter gene, DNA nuclear import was measured in cell cycle-arrested avian (DF-1) as well as human (HeLa) and mouse cells. The results showed efficient accumulation of nuclear forms of ASV DNA in gamma-irradiation-arrested cells. Efficient transduction of a GFP reporter gene was also observed after infection of cells that were arrested with gamma-irradiation, mitomycin C, nocodazole, or aphidicolin, confirming that nuclear import and integration of ASV DNA can occur in the absence of mitosis. By monitoring GFP



expression in individual cells, we also obtained evidence for nuclear import of viral DNA during interphase in cycling cells. Lastly, we observed that ASV can transduce postmitotic mouse neurons. These results support an active nuclear import mechanism for the oncoretrovirus ASV and suggest that this mechanism can operate in both nondividing and dividing cells.

CC Cytology - General 02502  
 Cytology - Animal 02506  
 Cytology - Human 02508  
 Biochemistry studies - General 10060  
 Biochemistry studies - Nucleic acids, purines and pyrimidines 10062  
 Biochemistry studies - Proteins, peptides and amino acids 10064  
 Enzymes - General and comparative studies: coenzymes 10802  
 Nervous system - Physiology and biochemistry 20504  
 Genetics of bacteria and viruses 31500  
 Virology - Animal host viruses 33506  
 Medical and clinical microbiology - Virology 36006

IT Major Concepts  
 Biochemistry and Molecular Biophysics; Cell Biology; Infection

IT Parts, Structures, & Systems of Organisms  
 neurons: nervous system

IT Chemicals & Biochemicals  
 DNA; enzymes; green fluorescent protein reporter genes; integrases;  
 proteins; viral DNA: nuclear entry mechanisms

IT Miscellaneous Descriptors  
 cell division; interphase cells: viral transduction; mitosis; viral  
 genomes: integration

ORGN Classifier  
 Aves 85500  
 Super Taxa  
 Vertebrata; Chordata; Animalia  
 Organism Name  
 bird: host  
 Taxa Notes  
 Animals, Birds, Chordates, Nonhuman Vertebrates, Vertebrates

ORGN Classifier  
 Hominidae 86215  
 Super Taxa  
 Primates; Mammalia; Vertebrata; Chordata; Animalia  
 Organism Name  
 HeLa cell line  
 human  
 Taxa Notes  
 Animals, Chordates, Humans, Mammals, Primates, Vertebrates

ORGN Classifier  
 Muridae 86375  
 Super Taxa  
 Rodentia; Mammalia; Vertebrata; Chordata; Animalia  
 Organism Name  
 mouse  
 Taxa Notes  
 Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals,  
 Rodents, Vertebrates

ORGN Classifier  
 Retroviridae 03305  
 Super Taxa  
 DNA and RNA Reverse Transcribing Viruses; Viruses; Microorganisms  
 Organism Name  
 avian sarcoma virus: pathogen  
 retrovirus: pathogen  
 Taxa Notes  
 DNA and RNA Reverse Transcribing Viruses, Microorganisms, Viruses

RN 52350-85-3 (integrases)  
 L5 ANSWER 4 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
 DUPLICATE 3  
 AN 2001:538663 BIOSIS  
 DN PREV200100538663  
 TI Green fluorescent protein rendered susceptible to proteolysis: Positions  
 for protease-sensitive insertions.  
 AU Chiang, Cheng-Feng; Okou, David T.; Griffin, Tony B.; Verret, C. Reynold;  
 Williams, Myron N. V. [Reprint author]  
 CS Department of Chemistry, Clark Atlanta University, Atlanta, GA, 30314, USA  
 mnwill@cau.edu  
 SO Archives of Biochemistry and Biophysics, (October 15, 2001) Vol. 394, No.  
 2, pp. 229-235. print.  
 CODEN: ABBIA4. ISSN: 0003-9861.  
 DT Article  
 LA English  
 ED Entered STN: 21 Nov 2001  
 Last Updated on STN: 25 Feb 2002  
 AB The green fluorescent protein (**GFP**) is highly resistant to  
 proteolysis and remains uncleaved after prolonged incubation with trypsin  
 or pronase despite several putative tryptic and chymotryptic sites in  
 exposed loops. We have rendered **GFP** sensitive to proteolysis by  
 inserting five amino acids, IEGRS, in loops at position 157,  
 172, or 189. Excitation and emission maxima of the three insertion  
 mutants were similar to those of wild type, but quantum yields of mutants  
 OMEGA172 and OMEGA189 were lower, indicating increased freedom of the  
 fluorophore. Trypsin cleaved the native (folded) form of each mutant at a  
 unique site defined by the insert. Pronase also yields similar digestion  
 patterns in these variants, but further proteolysis was also observed,  
 suggesting that the primary cleavage relaxes **GFP** structure and  
 reveals previously inaccessible sites. Fluorescence of OMEGA189 changed  
 little upon digestion with trypsin but decreased progressively by as much  
 as 40% upon digestion with increasing amounts of pronase. Fluorescence of  
 other variants was not affected significantly by the proteases, further  
 confirming the remarkable stabilities of **GFP** variants. These  
 constructs define a new conformation-sensitive site around residue 189 of  
**GFP** and show that **GFP** may be useful for design of  
 protease-susceptible molecules for monitoring of specific proteolytic  
 activities in vivo.  
 CC Biochemistry studies - General 10060  
 Biochemistry studies - Proteins, peptides and amino acids 10064  
 Enzymes - General and comparative studies: coenzymes 10802  
 Physiology and biochemistry of bacteria 31000  
 IT Major Concepts  
 Biochemistry and Molecular Biophysics  
 IT Chemicals & Biochemicals  
 factor Xa; green fluorescent protein; pronase; protease; trypsin  
 IT Methods & Equipment  
 mutagenesis: analytical method, genetic engineering, genetic method  
 IT Miscellaneous Descriptors  
 protease-sensitive insertions; proteolysis  
 ORGN Classifier  
 Bacteria 05000  
 Super Taxa  
 Microorganisms  
 Organism Name  
 bacteria  
 Taxa Notes  
 Bacteria, Eubacteria, Microorganisms  
 RN 9002-05-5 (factor Xa)  
 9036-06-0 (pronase)

9001-92-7 (protease)  
9002-07-7 (trypsin)

L5 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN  
AN 2000:359911 CAPLUS  
DN 133:164324  
ED Entered STN: 31 May 2000  
TI Antiparallel Leucine Zipper-Directed Protein Reassembly: Application to  
the Green Fluorescent Protein  
AU Ghosh, Indraneel; Hamilton, Andrew D.; Regan, Lynne  
CS Department of Chemistry, Yale University, New Haven, CT, 06520, USA  
SO Journal of the American Chemical Society (2000), 122(23), 5658-5659  
CODEN: JACSAT; ISSN: 0002-7863  
PB American Chemical Society  
DT Journal  
LA English  
CC 34-4 (Amino Acids, Peptides, and Proteins)  
Section cross-reference(s): 6  
AB A general method for reassembly of protein fragments mediated by  
noncovalent association of antiparallel leucine zippers was described, using  
green fluorescent protein (GFP) from *Aequorea victoria* as an  
example. GFP was dissected between residues 157-158,  
a position that has been shown to accommodate a 20-residue amino acid  
insertion; the fragments were termed NGFP (N-terminal-containing) and  
CGFP (C-terminal-containing), resp. Two designed helices, NZ or CZ, were  
attached, NZ to the NGFP C-terminal by a six-residue linker, and CZ to the  
CGFP N-terminal by a four-residue linker, to generate fragments NZGFP and  
CZGFP, resp. Genes coding for NZGFP and CZGFP were cloned and expressed  
using standard methods, and the proteins isolated. Equimolar amts. of the  
NZGFP and CZGFP fragments were denatured and dialyzed, and the reassembled  
peptides were visibly green and had fluorescence excitations and emission  
spectra identical to that of parent GFP.  
ST antiparallel leucine zipper reassembly green fluorescent protein  
IT Fluorescence  
Protein folding  
Self-assembly  
(antiparallel leucine zipper-directed protein reassembly of green  
fluorescent protein)  
IT Peptides, properties  
Proteins, general, properties  
RL: PRP (Properties)  
(antiparallel leucine zipper-directed protein reassembly of green  
fluorescent protein)  
IT 288154-44-9P 288154-45-0P 288154-47-2P 288154-48-3P 288154-49-4P  
RL: BPN (Biosynthetic preparation); PRP (Properties); SPN (Synthetic  
preparation); BIOL (Biological study); PREP (Preparation)  
(antiparallel leucine zipper-directed protein reassembly of green  
fluorescent protein)  
RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD  
RE  
(1) Abedi, M; Nucleic Acid Res 1998, V26, P623 CAPLUS  
(2) Bagshaw, C; Spectrophotometry and spectrofluorimetry: A practical approach  
1987, P91  
(3) Baird, G; Proc Natl Acad Sci U S A 1999, V96, P11241 CAPLUS  
(4) Bryson, J; Science 1995, V270, P935 CAPLUS  
(5) Cotton, G; Chem Biol 1999, V6, PR247 CAPLUS  
(6) Cotton, G; J Am Chem Soc 1999, V121, P1100 CAPLUS  
(7) Gay, G; Biochemistry 1994, V33, P7957  
(8) Harbury, P; Nature 1994, V371, P80 CAPLUS  
(9) Johnsson, N; Proc Natl Acad Sci U S A 1994, V91, P10340 CAPLUS  
(10) Karimova, G; Proc Natl Acad Sci U S A 1998, V95, P5752 CAPLUS  
(11) Kohn, W; J Biol Chem 1997, V272, P2583 CAPLUS

- (12) Kraulis, P; J Appl Crystallog 1991, V24, P946
- (13) Krylov, D; EMBO J 1994, V13, P2849 CAPLUS
- (14) Lupas, A; Trends Biochem Sc 1996, V21, P375 CAPLUS
- (15) Miesenbock, G; Nature 1998, V394, P192 CAPLUS
- (16) Miyawaki, A; Nature 1997, V388, P882 CAPLUS
- (17) Monero, O; J Biol Chem 1993, V268, P19218
- (18) Muir, T; Proc Natl Acad Sci U S A 1998, V95, P6705 CAPLUS
- (19) Nobuhide, D; FEBS Lett 1999, V453, P305
- (20) Oakley, M; Biochemistry 1997, V36, P2544 CAPLUS
- (21) Oakley, M; Biochemistry 1998, V37, P12603 CAPLUS
- (22) Ormo, M; Science 1996, V273, P1392 CAPLUS
- (23) O'Shea, E; Curr Biol 1993, V3, P658 CAPLUS
- (24) Palm, G; Nat Struct Biol 1997, V4, P361 CAPLUS
- (25) Pelletier, J; Nat Biotechnol 1999, V17, P683 CAPLUS
- (26) Pelletier, J; Proc Natl Acad Sci U S A 1998, V95, P12141 CAPLUS
- (27) Regan, L; Curr Opin Struct Biol 1999, V9, P494 CAPLUS
- (28) Reid, B; Biochemistry 1997, V36, P6786 CAPLUS
- (29) Remy, I; Proc Natl Acad Sci U S A 1999, V96, P5394 CAPLUS
- (30) Richards, F; J Biol Chem 1959, V234, P1459 CAPLUS
- (31) Rossi, F; Proc Natl Acad Sci U S A 1997, V94, P8405 CAPLUS
- (32) Shiba, K; Proc Natl Acad Sci U S A 1992, V89, P1880 CAPLUS
- (33) Southworth, M; EMBO J 1998, V17, P918 CAPLUS
- (34) Topell, S; FEBS Lett 1999, V457, P283 CAPLUS
- (35) Tsien, R; Annu Rev Biochem 1998, V67, P509 CAPLUS
- (36) Xu, R; Proc Natl Acad Sci U S A 1999, V96, P388 CAPLUS
- (37) Yao, S; Nature 1998, V396, P447 CAPLUS
- (38) Zhang, T; Biochemistry 1993, V32, P12311 CAPLUS
- (39) Zutshi, R; Curr Opin Chem Biol 1998, V2, P62 CAPLUS

L5 ANSWER 6 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
DUPLICATE 4

AN 2000:533558 BIOSIS

DN PREV200000533558

TI Gene structure and expression study of the SEDL gene for  
spondyloepiphyseal dysplasia tarda.

AU Gecz, Jozef [Reprint author]; Hillman, Marie A.; Gedeon, Agi K.; Cox,  
Timothy C.; Baker, Elizabeth; Mulley, John C.

CS Department of Cytogenetics and Molecular Genetics, Women's and Children's  
Hospital, 72 King William Road, North Adelaide, SA, 5006:  
jgecz@medicine.adelaide.edu.au, Australia

SO Genomics, (October 15, 2000) Vol. 69, No. 2, pp. 242-251. print.  
CODEN: GNMCEP. ISSN: 0888-7543.

DT Article

LA English

ED Entered STN: 13 Dec 2000

Last Updated on STN: 11 Jan 2002

AB Spondyloepiphyseal dysplasia tarda (SEDL) is an X-linked recessive  
disorder of endochondral bone formation caused by mutations in the SEDL  
gene. Here we present the structural analysis and subcellular  
localization of human SEDL. The SEDL gene is composed of six exons and  
spans a genomic region of approx 20 kb in Xp22. It contains four Alu  
sequences in its 3' UTR and an alternatively spliced MER20 sequence in its  
5' UTR (exon 2). Complex alternative splicing was detected for exon 4.  
Altogether seven SEDL pseudogenes were detected in the human genome:  
SEDLP1, a transcribed retropseudogene (or retro-xaptonuon) on chromosome  
19q13.4 with potential to encode a protein identical to that of the SEDL  
gene; SEDLP2, another retropseudogene (not transcribed) on chromosome 8;  
and five truncated pseudogenes, SEDLP3-SEDL7, on chromosome Yq11.23.  
Based on the knowledge of the yeast SEDL ortholog we speculated that the  
SEDL protein may participate along the ER-to-Golgi transport compartments.  
To test this hypothesis we performed transient transfection studies with  
tagged recombinant mammalian SEDL proteins in Cos-7 cells. The tagged

SEDL proteins localized to perinuclear structures that partly overlapped with the intermediate ER-Golgi compartment (ERGIC; or vesicular tubular complex, VTC). Two human SEDL mutations (157-158delAT and C271T(STOP)) introduced into SEDL FLAG and GFP constructs led to the misplacement of the SEDL protein primarily to the cell nucleus and partially to the cytoplasm. Based on these experiments we suggest that the COOH end of the SEDL protein might be responsible for proper targeting of SEDL along the ER-Golgi membrane compartments (including Golgi and ERGIC/VTC).

```
CC  Bones, joints, fasciae, connective and adipose tissue - Pathology  18006
    Cytology - Animal  02506
    Cytology - Human  02508
    Genetics - General  03502
    Genetics - Animal  03506
    Genetics - Human  03508
    Bones, joints, fasciae, connective and adipose tissue - Physiology and
    biochemistry  18004
    Development and Embryology - Pathology  25503
IT  Major Concepts
    Molecular Genetics (Biochemistry and Molecular Biophysics); Skeletal
    System (Movement and Support)
IT  Parts, Structures, & Systems of Organisms
    X chromosome
IT  Diseases
    spondyloepiphyseal dysplasia tarda: bone disease, congenital disease,
    X-linked recessive disorder
IT  Miscellaneous Descriptors
    human genome
ORGN Classifier
    Cercopithecidae  86205
    Super Taxa
    Primates; Mammalia; Vertebrata; Chordata; Animalia
    Organism Name
    Cos-7 cell line
    Taxa Notes
    Animals, Chordates, Mammals, Nonhuman Mammals, Nonhuman Vertebrates,
    Nonhuman Primates, Primates, Vertebrates
GEN  human SEDL gene (Hominidae): expression study, gene structure, subcellular
    localization; human SEDLP1 pseudogene (Hominidae): expression study, gene
    structure, subcellular localization
```

=> d his

(FILE 'HOME' ENTERED AT 15:24:06 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT  
15:24:34 ON 26 APR 2004

```
L1      19 S GFP AND 157
L2      24 S GFP AND 172
L3      4 S L1 AND L2
L4      1 DUPLICATE REMOVE L3 (3 DUPLICATES REMOVED)
L5      6 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)
```

=> duplicate remove l2

DUPLICATE PREFERENCE IS 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT'  
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n  
PROCESSING COMPLETED FOR L2

```
L6      11 DUPLICATE REMOVE L2 (13 DUPLICATES REMOVED)
```

=> d l6 1-11 all

L6 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN  
 AN 2003:855957 CAPLUS  
 DN 139:347345  
 ED Entered STN: 31 Oct 2003  
 TI Functional complementation of two green fluorescent protein fragments and  
 their use in a method for detecting protein - protein interactions and  
 drug screening  
 IN Dahl, Soren Weis; Terry, Bernard Robert  
 PA Bioimage A/S, Den.  
 SO PCT Int. Appl., 96 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 IC ICM C07K014-435  
 ICS G01N033-68  
 CC 6-3 (General Biochemistry)  
 Section cross-reference(s): 1, 3, 9  
 FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2003089464	A1	20031030	WO 2002-DK882	20021219
	W:				AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ
	RW:				GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
	US 2003219717	A1	20031127	US 2003-370570	20030224
	WO 2003089627	A1	20031030	WO 2003-DK266	20030422
	W:				AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG
	RW:				GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRAI	DK 2002-591	A	20020419		
	DK 2002-896	A	20020613		
	DK 2002-1029	A	20020701		
	WO 2002-DK882	A1	20021219		

AB Fluorescence complementation products with intensity levels mimicking the full length intensities are obtained by introduction of improved folding capabilities with a mutation in position preceding the chromophore. The present application discloses that certain Green Fluorescent Proteins (**GFPs**) can be reassembled and form a functional fluorescent protein when expressed as two independent proteins halves. For example, when EGFP is expressed in mammalian cells, choosing a split site located in a loop region between the residues that form the beta-sheet structures of the **GFP** beta-barrel results in intense fluorescence. An additive increase is obtained by splitting the **GFP** between amino acids 172 and 173. The present application further illustrates that yellow variant of **GFP**, EYFP, is also reassembled and, surprisingly, the fluorescence from the reassembled protein is markedly

enhanced if it contains the F64L mutation. The reassembly of proteins does not occur if the two independent proteins halves are fused to non-interacting proteins. But, when brought together, they are reassembled to form a functional fluorescent protein. Screening for drugs capable of preventing interaction between proteins is performed by selecting the cells with the highest dynamic range through Fluorescence Activated Cell Sorting (FACS), as illustrated with the ability of FK506 to break the rapamycin induced interaction between FRB and FKBP.

- ST green fluorescent protein fragment functional complementation protein interaction detection; **GFP** fragment functional complementation protein interaction detection drug screening
- IT Cytometry
  - (FACS (fluorescence-activated cell sorting); functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)
- IT Renilla
  - (**GFP** from; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)
- IT Proteins
  - RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
    - (cyan fluorescent, enhanced, ECFP; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)
- IT Plasmid vectors
  - (encoding N- or C-terminal half of **GFP**; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)
- IT Protein folding
  - (fluorescent, improved capabilities; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)
- IT Molecular cloning
  - (functional expression; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)
- IT Proteins
  - RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
    - (green fluorescent, **GFP**, mutated; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)
- IT Proteins
  - RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
    - (green fluorescent, enhanced, EGFP; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)
- IT Proteins
  - RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)
    - (interaction detection; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)
- IT Conformation
  - (loop, protein, of **GFP**, split site located in; functional complementation of two green fluorescent protein fragments and their

use in method for detecting protein - protein interactions and drug screening)

IT Animal cell  
(mammalian, protein - protein interactions in; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Chromophores  
(mutated amino acid preceding; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Drug screening  
(of modulators of protein-protein interaction; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Proteins  
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(red fluorescent, DsRed; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT Animal cell line  
(stably expressing heterologous conjugate comprising protein of interest, establishing of; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions)

IT Proteins  
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(yellow fluorescent, enhanced, EYFP; functional complementation of two green fluorescent protein fragments and their use in method for detecting protein - protein interactions and drug screening)

IT 618126-07-1  
RL: PRP (Properties)  
(Unclaimed; functional complementation of two green fluorescent protein fragments and their use in a method for detecting protein - protein interactions and drug screening)

IT 618126-03-7, 7: PN: WO03089464 SEQID: 7 unclaimed DNA 618126-05-9, 9: PN: WO03089464 SEQID: 9 unclaimed DNA 618126-08-2 618126-09-3  
618126-10-6 618126-11-7 618126-12-8 618126-13-9 618126-14-0  
618126-15-1 618126-16-2 618126-17-3 618126-18-4 618126-19-5  
618126-20-8 618126-21-9 618126-22-0 618126-23-1 618126-24-2  
618126-25-3 618126-26-4 618126-27-5 618126-28-6 618126-29-7  
618126-30-0 618126-31-1 618126-32-2 618126-33-3 618126-34-4  
618126-35-5 618126-36-6 618126-37-7 618126-38-8 618126-39-9  
618126-40-2 618126-41-3 618126-42-4 618126-43-5  
RL: PRP (Properties)  
(unclaimed nucleotide sequence; functional complementation of two green fluorescent protein fragments and their use in a method for detecting protein - protein interactions and drug screening)

IT 618125-97-6 618125-98-7 618125-99-8 618126-00-4 618126-01-5  
618126-02-6 618126-04-8 618126-06-0  
RL: PRP (Properties)  
(unclaimed protein sequence; functional complementation of two green fluorescent protein fragments and their use in a method for detecting protein - protein interactions and drug screening)

IT 618070-80-7 618070-81-8  
RL: PRP (Properties)  
(unclaimed sequence; functional complementation of two green



fluorescent protein fragments and their use in a method for detecting protein - protein interactions and drug screening)

RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD  
RE

- (1) Hamilton, A; WO 0187919 A 2001 CAPLUS
- (2) Japan Science & Tech Corp; EP 1229330 A 2002 CAPLUS
- (3) Nagai, T; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 2001, V98(6), P3197 CAPLUS
- (4) Ozawa, T; ANALYTICAL CHEMISTRY 2000, V72(21), P5151 CAPLUS
- (5) Ozawa, T; ANALYTICAL CHEMISTRY 2001, V73(24), P5866 CAPLUS
- (6) Palm, G; NATURE STRUCTURAL BIOLOGY 1997, V4(5), P361 CAPLUS
- (7) Stratagene Inc; WO 0164843 A 2001 CAPLUS

L6 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

AN 2003:203300 CAPLUS

DN 138:232974

ED Entered STN: 14 Mar 2003

TI Method for production of mutant library of protein with various sizes and sequences using PCR-coupled recombination or sequence-directed recombination

IN Kim, Hak Sung; Kim, Geun Joong; Cheon, Young Hoon; Lee, Dong Eun

PA Korea Advanced Institute of Science and Technology, S. Korea

SO U.S. Pat. Appl. Publ., 12 pp.

CODEN: USXXCO

DT Patent

LA English

IC C12Q001-68; G01N033-53; C12P019-34; C12P021-02; C12N005-06; C07K014-00

NCL 435007100; 435069100; 435091200; 435252300; 530350000

CC 3-2 (Biochemical Genetics)

Section cross-reference(s): 6, 7, 10

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2003049686	A1	20030313	US 2002-112455	20020329
	JP 2003102484	A2	20030408	JP 2002-89976	20020327
	DE 10214653	A1	20030327	DE 2002-10214653	20020403
PRAI	KR 2001-55394	A	20010910		

AB The invention provides a method for manufacturing a mutant library of proteins with various sizes and sequences from a parental protein. The invention also provides microorganisms transformed with plasmid vectors containing a recombinant gene prepared by insertion of genomic DNA into a defective template (gene). The invention relates that said insertion of genomic DNA into defective gene is carried out by PCR-coupled recombination or sequence-directed recombination employing a ligase. The invention further provides a process for obtaining said proteins using said transformed microorganisms. Specifically, the invention prepared several defective green fluorescent protein (GFP) genes by deletion of nucleotide sequences encoding amino acids of GFPuv, and prepared a defective dihydroorotase gene by deletion of dozens of nucleotides. Recombinant DNAs were constructed by inserting Escherichia coli genomic DNA fragments into the BamHI site of defective genes. A library of microorganisms transformed with plasmids containing said recombinant DNAs was constructed.

ST mutant protein library generation PCR recombination sequence directed; sequence Escherichia peptide insertion defective GFP gene; green fluorescent protein gene defective Escherichia genomic DNA recombination; dihydroorotase gene defective Escherichia genomic DNA recombination

IT PCR (polymerase chain reaction)

(-coupled recombination; use of site-directed mutagenesis and PCR-coupled recombination for generation of proteins with various sizes and sequences)

IT Genetic element

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES

(Uses)

(BamHI restriction enzyme recognition site; sequences of various Escherichia coli peptides inserted into BamHI recognition site of defective **GFP** genes, which results in fluorescent proteins of various lengths)

IT Fusion proteins (chimeric proteins)

RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)

(E. coli peptides fused to dihydroorotase; use of site-directed mutagenesis and PCR-coupled recombination for generation of protein with various sizes and sequences having dihydroorotase activity)

IT Escherichia coli

(JM109/pMAL-c2/gfpI5 (KCTC 10058BP), transformed; Escherichia coli transformed with recombinant plasmid vectors containing defective **GFP** genes linked to genomic E. coli DNA)

IT Escherichia coli

(JM109/pMAL-c2/gfpS22 (KCTC 10059BP), transformed; Escherichia coli transformed with recombinant plasmid vectors containing defective **GFP** genes linked to genomic E. coli DNA)

IT Fusion proteins (chimeric proteins)

RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)

(fluorescent, E. coli peptides fused to **GFP**; use of site-directed mutagenesis, PCR-coupled recombination, and/or sequence-directed recombination using ligase for generation of fluorescent proteins with various sizes and sequences)

IT Gene, animal

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(for defective **GFP**, **GFP** $\Delta$  172

-3/176(+2); defective **GFP** genes combined with genomic DNA from Escherichia coli, and use of said recombinant DNA in production of fluorescent proteins of various sizes and sequences)

IT Gene, animal

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(for defective **GFP**, **GFP** $\Delta$ 176(+2); defective

**GFP** genes combined with genomic DNA from Escherichia coli, and use of said recombinant DNA in production of fluorescent proteins of various sizes and sequences)

IT Gene

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(for defective dihydroorotase, DHO $\Delta$ 68-70(+1); defective dihydroorotase gene, and its linkage to Escherichia coli genomic DNA for use in production of proteins with various sizes and sequences possessing dihydroorotase activity)

IT Proteins

RL: BSU (Biological study, unclassified); BIOL (Biological study) (green fluorescent; use of site-directed mutagenesis, PCR-coupled recombination, and/or sequence-directed recombination using ligase for generation of fluorescent proteins with various sizes and sequences)

IT Plasmid vectors

(microorganisms transformed with recombinant plasmid vectors containing defective **GFP** genes linked to genomic Escherichia coli DNA, or defective dihydroorotase gene lined to E. coli DNA)

IT Peptide library

(mutant; method for production of mutant library of protein with various sizes and sequences using PCR-coupled recombination or sequence-directed recombination)

IT Nucleic acid library

(of recombinant DNAs; microorganisms transformed with recombinant

plasmid vectors containing defective **GFP** genes linked to genomic Escherichia coli DNA, or defective dihydroorotase gene lined to E. coli DNA)

- IT Recombination, genetic  
(sequence-directed recombination using ligase; use of site-directed mutagenesis and sequence-directed recombination using ligase for generation of fluorescent proteins with various sizes and sequences)
- IT Protein sequences  
(sequences of various Escherichia coli peptides inserted into BamHI recognition site of defective **GFP** genes, which results in fluorescent proteins of various lengths)
- IT Mutagenesis  
(site-directed, deletion; use of site-directed mutagenesis, PCR-coupled recombination, and/or sequence-directed recombination using ligase for generation of proteins with various sizes and sequences)
- IT Microorganism  
(transformed; microorganisms transformed with recombinant plasmid vectors containing defective **GFP** genes linked to genomic Escherichia coli DNA, or defective dihydroorotase gene lined to E. coli DNA)
- IT Molecular cloning  
(use of site-directed mutagenesis, PCR-coupled recombination, and/or sequence-directed recombination using ligase for generation of proteins with various sizes and sequences)
- IT 501644-98-0 501644-99-1 501645-00-7 501645-01-8 501645-02-9  
501645-03-0 501645-04-1 501645-05-2 501645-06-3 501645-07-4  
501645-08-5 501730-73-0 501730-74-1 501730-75-2 501730-76-3  
501730-77-4 501730-78-5  
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
(amino acid sequence; sequences of various Escherichia coli peptides inserted into BamHI recognition site of defective **GFP** genes, which results in fluorescent proteins of various lengths)
- IT 37353-39-2, Polynucleotide ligase  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
(sequence-directed recombination using ligase; use of site-directed mutagenesis and sequence-directed recombination using ligase for generation of fluorescent proteins with various sizes and sequences)
- IT 9024-93-5, Dihydroorotase  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(use of site-directed mutagenesis and PCR-coupled recombination for generation of proteins with various sizes and sequences having dihydroorotase activity)

L6 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

AN 2003:296076 CAPLUS

DN 138:315791

ED Entered STN: 17 Apr 2003

TI Fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or  $\beta$ -lactamase

IN Anderson, David; Peelle, Beau Robert; Bogenberger, Jakob Maria

PA Rigel Pharmaceuticals, Inc., USA

SO U.S., 63 pp., Cont.-in-part of U.S. 6,180,343.

CODEN: USXXAM

DT Patent

LA English

IC ICM C07K004-00

ICS C07K014-435; C12Q001-68; C12N015-63; C12N015-12

NCL 530300000; 530350000; 435006000; 435320100; 536023400; 536023500

CC 3-1 (Biochemical Genetics)

Section cross-reference(s): 9

## FAN.CNT 4

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6548632	B1	20030415	US 1999-415765	19991008
	US 6180343	B1	20010130	US 1998-169015	19981008
	US 6548249	B1	20030415	US 2000-626581	20000727
	US 6562617	B1	20030513	US 2000-626580	20000727
	US 2001003650	A1	20010614	US 2000-749959	20001227
	US 6596485	B2	20030722		
	US 2003143562	A1	20030731	US 2002-177725	20020620
	US 2003224412	A1	20031204	US 2003-393449	20030318
PRAI	US 1998-169015	A2	19981008		
	US 1999-415765	A3	19991008		
	US 2002-177725	A2	20020620		
AB	The invention relates to the use of scaffold proteins, particularly green fluorescent protein (GFP) and $\beta$ -lactamase TEM-1, in fusion constructs with random and defined peptides and peptide libraries. The fusions act to increase the cellular expression levels, decrease the cellular catabolism, increase the conformational stability relative to linear peptides, and increase the steady state concns. of the random peptides and random peptide library members expressed in cells for the purpose of detecting the presence of the peptides and screening random peptide libraries. N-terminal, C-terminal, dual N- and C-terminal, and one or more internal fusions are all contemplated. Internal fusions in Renilla GFP may be made in loops 1-5 (amino acid residues 130-135, 154-159, 172-175, 188-193, or 208-216) for optimal presentation of the peptide. Inclusion of multiple highly flexible amino acid residues between GFP and the library allows minimal conformational constraints on the GFP. Designed insertion sites in loops 2-4 retain a high level of GFP fluorescence when the inserts are flanked by multiple glycines in the tetrapeptide linkers. Novel fusions utilizing self-binding peptides to create a conformationally stabilized fusion domain are also contemplated.				
ST	scaffold protein fusion random peptide library; green fluorescent protein fusion random peptide library; lactamase fusion random peptide library				
IT	Animal cell line (293, GFP fusions expressed in; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or $\beta$ -lactamase)				
IT	Aequorea Renilla reniformis (GFP from; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or $\beta$ -lactamase)				
IT	Animal cell line (JURKAT, GFP fusions expressed in; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or $\beta$ -lactamase)				
IT	Antigen presentation Peptide library (fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or $\beta$ -lactamase)				
IT	Fusion proteins (chimeric proteins) RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses) (fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or $\beta$ -lactamase)				
IT	Linking agents (glycine-based; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or $\beta$ -lactamase)				
IT	Proteins RL: ARU (Analytical role, unclassified); BUU (Biological use,				

unclassified); ANST (Analytical study); BIOL (Biological study); USES  
(Uses)  
(green fluorescent, enhanced; fusions of random peptide libraries in  
scaffold proteins such as green fluorescent protein or  
 $\beta$ -lactamase)

IT Proteins  
RL: ARU (Analytical role, unclassified); BUU (Biological use,  
unclassified); ANST (Analytical study); BIOL (Biological study); USES  
(Uses)  
(green fluorescent; fusions of random peptide libraries in scaffold  
proteins such as green fluorescent protein or  $\beta$ -lactamase)

IT Fluorescence  
(of **GFP** fusions in loops 2-4 with glycine-based linkers;  
fusions of random peptide libraries in scaffold proteins such as green  
fluorescent protein or  $\beta$ -lactamase)

IT Proteins  
RL: ARU (Analytical role, unclassified); BUU (Biological use,  
unclassified); ANST (Analytical study); BIOL (Biological study); USES  
(Uses)  
(scaffolding; fusions of random peptide libraries in scaffold proteins  
such as green fluorescent protein or  $\beta$ -lactamase)

IT Escherichia coli  
( $\beta$ -lactamase TEM-1 from; fusions of random peptide libraries in  
scaffold proteins such as green fluorescent protein or  
 $\beta$ -lactamase)

IT 511353-43-8  
RL: PRP (Properties)  
(Unclaimed; fusions of random peptide libraries in scaffold proteins  
such as green fluorescent protein or  $\beta$ -lactamase)

IT 9073-60-3,  $\beta$ -Lactamase  
RL: ARU (Analytical role, unclassified); BUU (Biological use,  
unclassified); ANST (Analytical study); BIOL (Biological study); USES  
(Uses)  
(fusions of random peptide libraries in scaffold proteins such as green  
fluorescent protein or  $\beta$ -lactamase)

IT 76862-68-5, GenBank V00365 136110-02-6, GenBank M58472 138364-37-1,  
GenBank M60466 138390-43-9, GenBank M65067 139782-89-1, GenBank M15035  
139784-89-7, GenBank X02872 139784-99-9, GenBank M15077 139786-07-5,  
GenBank M25346 139789-49-4, GenBank M26194 139805-89-3, GenBank J00140  
139855-08-6, GenBank J00060 139863-26-6, GenBank M86443 140002-89-7,  
GenBank K01193 140003-58-3, GenBank M15826 140006-57-1, GenBank M32108  
140006-61-7, GenBank M10961 140006-78-6, GenBank M55977 140026-45-5,  
GenBank M16446 140039-08-3, GenBank M11310 140055-20-5, GenBank M29942  
140055-22-7, GenBank M29941 140055-23-8, GenBank M29943 140060-13-5,  
GenBank K02611 140247-47-8, GenBank J01609 140252-19-3, GenBank M22126  
140259-58-1, GenBank M25666 140273-28-5, GenBank J04711 140294-17-3,  
GenBank J03398 140317-11-9, GenBank M60508 140558-28-7, GenBank M13128  
140718-03-2, GenBank J01636 140721-25-1, GenBank J01804 140740-40-5,  
GenBank M38561 140747-82-6, GenBank M34158 140770-54-3, GenBank J04792  
140787-38-8, GenBank M62917 140794-75-8, GenBank V00372 141145-61-1,  
GenBank M26434 141683-91-2, GenBank X58640 144623-65-4, GenBank L04970  
145258-01-1, GenBank L06280 147321-20-8, GenBank S48276 148939-74-6,  
GenBank S61961 151347-01-2, GenBank X74948 151548-08-2, GenBank L25411  
151579-06-5, GenBank U03059 152077-65-1, GenBank U03687 152281-89-5,  
GenBank L26954 152851-60-0, GenBank U04894 153317-13-6, GenBank L26316  
158536-81-3, GenBank L36849 163474-68-8, GenBank Z48231 163539-78-4,  
GenBank L39929 164478-57-3, GenBank L39928 164950-38-3, GenBank U16781  
167206-75-9, GenBank U31240 167714-65-0, GenBank U28961 170048-89-2,  
GenBank U36394 173710-42-4, GenBank L76273 181613-99-0, GenBank U61765  
182910-13-0, GenBank U62625 183049-69-6, GenBank U75991 183049-70-9,  
GenBank U75992 183100-65-4, GenBank Z75185 194082-52-5, GenBank U82227  
195650-72-7, GenBank AF012551 196718-14-6, GenBank AF085332

200762-49-8, GenBank AF016891 201951-34-0, GenBank AF025747  
 203605-36-1, GenBank U28239 205361-40-6, GenBank AJ227752 207106-77-2,  
 GenBank AB013080 207888-26-4, GenBank AF061748 209138-47-6, GenBank  
 AF060886 210450-36-5, GenBank AF072538 211159-07-8, GenBank AF073995  
 224328-20-5, GenBank U62928 225581-17-9, GenBank AB025433 235408-30-7,  
 GenBank AF170105 235740-40-6, GenBank AF173835 383838-60-6, GenBank  
 AF031307 384417-75-8, GenBank M37124 384427-77-4, GenBank M63501  
 384448-90-2, GenBank M57579 384452-21-5, GenBank J00423 384578-31-8,  
 GenBank D83710 384598-54-3, GenBank D25416 384598-55-4, GenBank E08320  
 384662-78-6, GenBank U51019 384971-65-7, GenBank E05448 385206-41-7,  
 GenBank U62931 389335-68-6, GenBank L37442 389733-83-9, GenBank U89490  
 392014-96-9, GenBank AF016535 392022-53-6, GenBank U62930 398096-93-0,  
 GenBank L07486

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)

(fusions of random peptide libraries in scaffold proteins such as green  
 fluorescent protein or  $\beta$ -lactamase)

IT 511353-44-9 511353-46-1

RL: PRP (Properties)

(unclaimed nucleotide sequence; fusions of random peptide libraries in  
 scaffold proteins such as green fluorescent protein or  
 $\beta$ -lactamase)

IT 511353-32-5 511353-33-6 511353-34-7 511353-35-8 511353-36-9  
 511353-37-0 511353-38-1 511353-39-2 511353-40-5 511353-41-6  
 511353-42-7 511353-45-0 511353-47-2

RL: PRP (Properties)

(unclaimed protein sequence; fusions of random peptide libraries in  
 scaffold proteins such as green fluorescent protein or  
 $\beta$ -lactamase)

IT 76869-17-5 95088-49-6 105468-12-0 110579-95-8 113516-56-6  
 115084-19-0 119766-62-0 122580-21-6 135941-52-5 206750-67-6  
 245759-04-0 245759-06-2 245759-08-4 246862-96-4 246862-97-5  
 246862-98-6 246862-99-7 246863-01-4 246863-03-6 246863-04-7  
 246863-05-8 246863-06-9 246863-07-0 246863-08-1 257933-92-9  
 260055-30-9 265979-95-1 265979-96-2 265979-97-3 265979-98-4  
 310404-89-8 475270-13-4 475270-18-9 511245-87-7 511245-90-2  
 511245-92-4 511245-94-6 511245-96-8 511245-98-0 511246-00-7  
 511246-14-3 511246-19-8 511246-35-8 511246-48-3 511246-51-8  
 511246-56-3 511246-61-0 511246-66-5

RL: PRP (Properties)

(unclaimed sequence; fusions of random peptide libraries in scaffold  
 proteins such as green fluorescent protein or  $\beta$ -lactamase)

RE.CNT 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

- (1) Abedi, M; Nucleic Acids Research 1998, V26(2), P623 CAPLUS
- (2) Adachi; J Biol Chem 1991, V266(5), P3186 CAPLUS
- (3) Anon; WO 9534664 1995 CAPLUS
- (4) Anon; WO 9711094 1997 CAPLUS
- (5) Anon; WO 9745538 1997 CAPLUS
- (6) Anon; WO 9836097 1998 CAPLUS
- (7) Anon; WO 9839483 1998 CAPLUS
- (8) Anon; WO 9924617 1999 CAPLUS
- (9) Anon; WO 9949019 1999 CAPLUS
- (10) Anon; WO 9949019 1999 CAPLUS
- (11) Betz; Biochemistry 1996, V35(21), P6955 CAPLUS
- (12) Borjigin; Journal of Biological Chemistry 1994, V269(20), P14715 CAPLUS
- (13) Bouthors; Biochem J 1998, V330(Pt 3), P1443
- (14) Bryan; US 6232107 B1 2001 CAPLUS
- (15) Cepko; Current Protocols in Molecular Biology, 1998, V2, P9.9.1
- (16) Chalfie, M; Photochemistry and Photobiology 1995, V62(4), P651 CAPLUS
- (17) de Prat Gay; Biochemistry 1994, V33(25), P7957 MEDLINE
- (18) Doi; CMLS Cellular and Molecular Life Sciences 1998, V54, P394 CAPLUS

(19) Doig; Protein Sci 1995, V4(7), P1325 CAPLUS  
(20) Doig; Protein Sci 1997, V6(1), P147 CAPLUS  
(21) Graddis; Biochemistry 1993, V32(47), P12664 CAPLUS  
(22) Hellinga; J Mol Biol 1991, V222, P787 CAPLUS  
(23) Huang; Proc Natl Acad Sci USA 1997, V94(16), P8801 CAPLUS  
(24) Jackson; Biochemistry 1991, V30(43), P10428 CAPLUS  
(25) Jelsch; Proteins 1993, V16(4), P364 CAPLUS  
(26) Kamb; US 6025485 A 2000 CAPLUS  
(27) Knox; Protein Eng 1993, V6(1), P11 CAPLUS  
(28) Ladner; US 5223409 A 1993 CAPLUS  
(29) Lander; TIBTECH 1995, V13, P426  
(30) Legendre; Nat Biotechnol 1999, V17(1), P67 CAPLUS  
(31) Longstaff; Biochemistry 1990, V29(31), P7339 CAPLUS  
(32) Lyu; Science 1990, V250(4981), P669 CAPLUS  
(33) Marshall; Diagn Microbiol Infect Dis 1995, V22(4), P353 CAPLUS  
(34) McConnell; J Mol Biol 1995, V250, P460 CAPLUS  
(35) McPhalen; Biochemistry 1987, V26(1), P261 CAPLUS  
(36) Monera; J Biol Chem 1993, V268(26), P19218 CAPLUS  
(37) Norman; Science 1999, V285(5427), P591 CAPLUS  
(38) O'Neil; Science 1990, V250(4981), P646 CAPLUS  
(39) Raquet; Proteins 1995, V23(1), P63 CAPLUS  
(40) Richardson; Science 1998, V240(4859), P1648  
(41) Rodrigues; Chem Biol 1995, V2(4), P223 CAPLUS  
(42) Ward; The Journal of Biological Chemistry 1979, V254(3), P781 CAPLUS  
(43) Yang; Nature Biotechnology 1996, V14, P246  
(44) Zlokarnik; Science 1998, V279(5347), P84 CAPLUS

L6 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
AN 2004:167884 BIOSIS  
DN PREV200400161946  
TI Angiogenesis in an in vivo model of adipose tissue development.  
AU Neels, Jaap G. [Reprint Author]; Loskutoff, David J. [Reprint Author]  
CS Cell Biology, Division of Vascular Biology, Scripps Research Institute, La Jolla, CA, USA  
SO Blood, (November 16 2003) Vol. 102, No. 11, pp. 148b. print.  
Meeting Info.: 45th Annual Meeting of the American Society of Hematology. San Diego, CA, USA. December 06-09, 2003. American Society of Hematology. CODEN: BLOOAW. ISSN: 0006-4971.  
DT Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LA English  
ED Entered STN: 24 Mar 2004  
Last Updated on STN: 24 Mar 2004  
AB Obesity is approaching epidemic levels in Westernized societies, and is associated with an increased risk for cardiovascular disease and cancer. Angiogenesis is a critical component of these pathological processes, and the expanding adipose tissue represents one of the few sites of active angiogenesis in the adult. In spite of the fundamental and clinical importance of angiogenesis in obesity and its associated pathologies, little is known about underlying mechanisms. Although angiogenesis is, historically, the most widely studied mechanism of blood vessel formation, it is now clear that other processes (e.g. endothelial progenitor cell (EPC) recruitment) may also contribute. Angiogenesis is characterized by endothelial sprouting from preexisting blood vessels and it relies on the proliferation, migration, and remodeling of fully differentiated endothelial cells (ECs). In contrast, EPCs are present in the peripheral blood of adult mammals, including humans, and these bone marrow derived EPCs are recruited to target organs and also appear to contribute to the formation of new vessels (vasculogenesis). The absence of suitable animal models has hindered attempts to delineate the importance of these, and possibly other mechanisms of neovascularization during adipose tissue development. In this report, we demonstrate that the 3T3-F442A model of

adipose tissue development (Green, H. and Kehinde, O., J. Cell Physiol. (1979) 101:169-172) may be a particularly useful in vivo model to study angiogenesis during adipogenesis. In this model, 3T3-F442A preadipocytes are implanted subcutaneously into athymic Balb/c nude mice. We show that these cells develop into highly vascularized fat pads over the next 14-21 days, and that these fat pads are morphologically indistinguishable from normal adipose tissue. Histological studies demonstrate that a new microvasculature is evident as early as 5 days after cell implantation, and real-time quantitative RT-PCR analyses show that the expression of endothelial markers (e.g. PECAM-1, FLT-4, TIE-1, and TIE-2) and adipogenesis markers (lipoprotein lipase and adipsin) increase in parallel during fat pad development. These results suggest that invasion of endothelial cells, and neovascularization of the fat pads occurs in parallel to adipogenesis during fat pad development. To investigate the potential contribution of EPCs to neovascularization in this model, we grew fat pads in athymic nude mice that had previously received a bone marrow transplantation from transgenic TIE2-GFP donor mice. Resulting fat pads were harvested at various times after injection and were stained for PECAM-1 to visualize the vasculature, and for GFP to determine the degree of EPC incorporation into it. A PECAM-1 positive neovasculature was observed in the developing fat pads from day 5 on. In spite of this, no GFP positive cells were observed in the various sections, the rare exception being those from 14-day old fat pads. The 14-day old fat pads showed a few GFP positive endothelial cells lining part of some vessel walls even though analysis by PECAM-1 staining established the presence of an abundant neovasculature. These preliminary studies suggest that the neovasculature originates by sprouting from preexisting, nerve-associated blood vessels located adjacent to the fat pad, and that endothelial progenitor cells only play a minor role in this process.

- CC General biology - Symposia, transactions and proceedings 00520
- Cytology - Animal 02506
- Enzymes - General and comparative studies: coenzymes 10802
- Metabolism - General metabolism and metabolic pathways 13002
- Nutrition - General studies, nutritional status and methods 13202
- Nutrition - Malnutrition and obesity 13203
- Cardiovascular system - Physiology and biochemistry 14504
- IT Major Concepts
  - Cardiovascular System (Transport and Circulation); Metabolism; Nutrition
- IT Parts, Structures, & Systems of Organisms
  - adipose tissue; endothelial progenitor cells: circulatory system; microvasculature: circulatory system; neovasculature: circulatory system
- IT Diseases
  - obesity: nutritional disease
  - Obesity (MeSH)
- IT Chemicals & Biochemicals
  - FLT-4: biomarker; PECAM-1: biomarker; TIE-1: biomarker; TIE-2: biomarker; adipsin: biomarker; lipoprotein lipase [EC 3.1.1.34]: biomarker
- IT Methods & Equipment
  - real-time RT-PCR [real-time reverse transcriptase-polymerase chain reaction]: genetic techniques, laboratory techniques
- IT Miscellaneous Descriptors
  - adipogenesis; angiogenesis; fat pad development
- ORGN Classifier
  - Muridae 86375
  - Super Taxa
    - Rodentia; Mammalia; Vertebrata; Chordata; Animalia
  - Organism Name
    - 3T3-F442A cell line (cell line)



Balb/C mouse (common): transgenic

Taxa Notes

Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals,  
Rodents, Vertebrates

RN 37213-56-2Q (adipsin)  
60296-01-7Q (adipsin)  
9004-02-8 (lipoprotein lipase)  
9004-02-8 (EC 3.1.1.34)

L6 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

AN 2002:895268 CAPLUS

DN 138:199159

ED Entered STN: 26 Nov 2002

TI Kallikreins when activating bradykinin B2 receptor induce its  
redistribution on plasma membrane

AU Hecquet, Claudie; Becker, Robert P.; Tan, Fulong; Erdos, Ervin G.

CS Department of Pharmacology, University of Illinois College of Medicine at  
Chicago, Chicago, IL, 60612, USA

SO International Immunopharmacology (2002), 2(13-14), 1795-1806  
CODEN: IINMBA; ISSN: 1567-5769

PB Elsevier Science B.V.

DT Journal

LA English

CC 2-10 (Mammalian Hormones)

AB The bradykinin (BK) B2 receptor (R) is directly activated by kallikreins  
and other serine proteases independent of BK release. Both the G $\alpha$ i  
and G $\alpha$ q proteins are involved, shown by the release of arachidonic  
acid and [Ca<sup>2+</sup>]<sub>i</sub> elevation. Site-directed mutagenesis of the receptor and  
the lack of heterogenous desensitization of the human B2R by the BK and  
kallikrein emphasize among others the differences between activation by  
the proteases and the peptide. To characterize further the mechanism  
whereby kallikreins activate and desensitize the B2R, the authors  
investigated the distribution of the human B2R tagged with the green  
fluorescent protein (B2-GFPct) on the plasma membrane of stably  
transfected Chinese hamster ovary (CHO) cells. The authors visualized the  
movement of B2-GFPct R with confocal fluorescence microscopy after  
activation by BK or a by serine protease. Continued exposure of the cells  
to BK led to B2R internalization within 15-20 min. Porcine pancreatic and  
human recombinant tissue kallikreins induced a rapid definite  
redistribution of receptors on the plasma membrane within 5 min, prior to  
internalization. These effects of kallikrein were blocked by the B2R  
antagonist HOE 140 and by the kallikrein inhibitor, aprotinin. The B2R  
was also activated by endoproteinases LysC and ArgC and trypsin, but these  
enzymes did not induce redistribution, only internalization. In control  
expts., kallikrein had no effect on cells transfected to stably express  
the angiotensin-converting enzyme-green fluorescent protein (GFP  
) . Thus, kallikreins when activating the BK B2R also trigger its  
redistribution on plasma membrane.

ST kallikrein bradykinin B2 receptor redistribution plasma membrane  
activation

IT Bradykinin receptors

RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(B2; kallikreins and other serine proteases redistribution of  
bradykinin B2 receptor plasma membrane in receptor activation and  
involved mechanisms)

IT Biological transport

(calcium; kallikreins and other serine proteases redistribution of  
bradykinin B2 receptor plasma membrane in receptor activation and  
involved mechanisms)

IT Artery

(endothelium; kallikreins and other serine proteases redistribution of  
bradykinin B2 receptor plasma membrane in receptor activation and

involved mechanisms)

IT Biological transport  
(internalization; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)

IT Cell membrane  
Human  
(kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)

IT Biological transport  
(redistribution; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)

IT 74-79-3, Arginine, biological studies  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(bradykinin B2 receptor residue 169, kallikrein cleavage site; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)

IT 56-87-1, Lysine, biological studies  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(bradykinin B2 receptor residue 172, kallikrein cleavage site; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)

IT 58-82-2, Bradykinin 506-32-1, Arachidonic acid 9001-01-8, Kallikrein 9002-07-7, Trypsin 37259-58-8, Serine protease 56645-49-9, Cathepsin G 63551-76-8, Phosphatidylinositol phospholipase C 123175-81-5, Endoproteinase ArgC 123175-82-6, Endoproteinase LysC 389069-73-2, Kallikrein 410538-33-9, Plasma Kallikrein  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)

IT 7440-70-2, Calcium, biological studies  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(transport; kallikreins and other serine proteases redistribution of bradykinin B2 receptor plasma membrane in receptor activation and involved mechanisms)

RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

- (1) Abe, M; Hypertension 1995, V26, P891 CAPLUS
- (2) Bachvarov, D; J Pharmacol Exp Ther 2001, V297, P19 CAPLUS
- (3) Bhoola, K; Pharmacol Rev 1992, V44, P1 CAPLUS
- (4) Bohm, S; News Physiol Sci 1998, V13, P231 CAPLUS
- (5) de Weerd, W; J Biol Chem 1997, V272, P17858 CAPLUS
- (6) Dery, O; Am J Physiol 1998, V274, PC1429 CAPLUS
- (7) Erdos, E; Handb Exp Pharmacol 1979, VXXV, P1
- (8) Feletou, M; Br J Pharmacol 1988, V93, P515 CAPLUS
- (9) Gutowski, S; J Biol Chem 1991, V266, P20519 CAPLUS
- (10) Haasemann, M; J Cell Sci 1998, V111, P917 CAPLUS
- (11) Hecquet, C; Mol Pharmacol 2000, V58(4), P828 CAPLUS
- (12) Hess, J; Biochem Biophys Res Commun 1992, V184, P260 CAPLUS
- (13) Kassel, B; Biochem Biophys Res Commun 1965, V20(4), P463
- (14) Kraut, H; Hoppe-Seyler Z Physiol Chem 1930, V192, P1 CAPLUS
- (15) Liao, J; J Clin Invest 1993, V92, P2168 CAPLUS
- (16) Marcic, B; Hypertension 1999, V33, P835
- (17) Minshall, R; Circ Res 1997, V81, P848 CAPLUS
- (18) Molino, M; J Biol Chem 1994, V270, P11168
- (19) Rocha e Silva, M; Ann NY Acad Sci 1963, V104, P190
- (20) Salvesen, G; Handbook of proteolytic enzymes 1998, P60

- (21) Skidgel, R; Biochem Pharmacol 1991, V41, P1335 CAPLUS
- (22) Takada, Y; Biochem J 1985, V232, P851 CAPLUS
- (23) Werle, E; Biochem Z 1937, V289, P217 CAPLUS
- (24) Yamada, K; Kidney Int 1982, V22, P331 CAPLUS

L6 ANSWER 6 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
 DUPLICATE 1

AN 2002:219934 BIOSIS

DN PREV200200219934

TI Transient expression of fluorescent tau proteins promotes process  
 formation in PC12 cells: Contributions of the tau C-terminus to this  
 process.

AU Yu, Jiang-Zhou; Kuret, Jeff; Rasenick, Mark M. [Reprint author]

CS Department of Physiology and Biophysics, University of Illinois at Chicago  
 College of Medicine, 835 S. Wolcott Ave., Room E202, Chicago, IL,  
 60612-7342, USA  
 raz@uic.edu

SO Journal of Neuroscience Research, (March 1, 2002) Vol. 67, No. 5, pp.  
 625-633. print.  
 CODEN: JNREDK. ISSN: 0360-4012.

DT Article

LA English

ED Entered STN: 27 Mar 2002  
 Last Updated on STN: 27 Mar 2002

AB The neuronal microtubule-associated protein, tau promotes microtubule  
 assembly and has been implicated in the development of axonal morphology.  
 In this study, PC12 cells were transiently transfected with constructs  
 coding fusion proteins of human tau with green fluorescent protein (  
**GFP**). Expression of tau constructs actively stabilized  
 microtubules. Expression of the C-terminus of tau can mimic this effect  
 in living cells, though to a lesser extent because of the absence of the  
 tau N-terminus. However, tau colocalization with microtubules did not  
 require the presence of the tau N-terminus. Transient expression of tau  
 (including tau24, a four-repeat human tau isoform encoded in 383 residues,  
 and tau23, human fetal tau isoform encoded in 352 residues) stimulated  
 process formation in PC12 cells, and this occurred faster with tau24 than  
 with tau23. The residues (residues 154-172 in tau23) that  
 confer microtubule nucleation activity of tau in vitro are not required  
 for tau-directed process formation. However, when tau induces the  
 formation of cellular processes in response to cortical breakdown by  
 cytochalasin B, residues 154-172 must be present. Thus, it  
 appears that tau may serve to promote cellular process outgrowth in  
 cultured neuronal cells and that C-terminus of tau is essential to this  
 process.

CC Cytology - Animal 02506  
 Muscle - Physiology and biochemistry 17504  
 Nervous system - Physiology and biochemistry 20504

IT Major Concepts  
 Nervous System (Neural Coordination)

IT Parts, Structures, & Systems of Organisms  
 microtubule: muscular system; neuronal cell: nervous system

IT Chemicals & Biochemicals  
 cytochalasin B; tau protein; tau23; tau24

ORGN Classifier  
 Muridae 86375  
 Super Taxa  
 Rodentia; Mammalia; Vertebrata; Chordata; Animalia

Organism Name  
 PC12 cell line

Taxa Notes  
 Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals,  
 Rodents, Vertebrates

RN 14930-96-2 (cytochalasin B)

L6 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

AN 2002:858987 CAPLUS

DN 138:149221

ED Entered STN: 13 Nov 2002

TI Development of a positive genetic selection system for inhibition of protein splicing using mycobacterial inteins in *Escherichia coli* DNA gyrase subunit A

AU Adam, Eric; Perler, Francine B.

CS New England BioLabs, Beverly, MA, 01915, USA

SO Journal of Molecular Microbiology and Biotechnology (2002), 4(5), 479-487  
CODEN: JMMBFF; ISSN: 1464-1801

PB Horizon Scientific Press

DT Journal

LA English

CC 6-3 (General Biochemistry)  
Section cross-reference(s): 3, 10

AB An intein-based pos. genetic selection system was developed to study protein splicing and to provide a selection system with the potential for finding splicing inhibitors. Inteins can be novel anti-microbial targets when present in essential proteins since blocking splicing would kill the organism. For example, pathogenic mycobacteria encode inteins that interrupt DNA gyrase. The gyrase selection system exploits (1) splicing of inteins out of Gyrase A and (2) the dominant lethal effect of quinolone poisoning of DNA gyrase, which in turn blocks replication. The system was adapted for whole-cell high-throughput screening using green fluorescent protein as an automatable readout of viability. To demonstrate the efficacy of this system, mutations that blocked splicing of the *Mycobacterium xenopi* Gyrase A intein were isolated. Splicing was then assayed at a second temperature to identify inteins with a temperature-sensitive splicing phenotype. Mutations were mapped onto a structure-based sequence alignment, which led to the rational prediction of a temperature-sensitive splicing mutation. GyrA intein subdomain relationships also provided insight into intein evolution.

ST *Mycobacterium* DNA gyrase A intein protein splicing mutant screening; protein intein splicing inhibitor genetic selection *Escherichia* gene *gyrA*

IT Enzymes, biological studies  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
(DNA gyrases; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in *Escherichia coli* DNA gyrase subunit A)

IT *Escherichia coli*  
High throughput screening  
*Mycobacterium flavescens*  
*Mycobacterium gordonae*  
*Mycobacterium kansasii*  
*Mycobacterium leprae*  
*Mycobacterium mageritense*  
*Mycobacterium xenopi*  
Protein splicing  
(development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in *Escherichia coli* DNA gyrase subunit A)

IT Inteins  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in *Escherichia coli* DNA gyrase subunit A)

IT Growth, microbial

(development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and dominant lethal effect of quinonolones on gyrase)

IT Reporter gene  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (for **GFP** (green fluorescent protein); development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A)

IT Proteins  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (green fluorescent, reporter; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A)

IT Gene, microbial  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (gyrA96, ofloxacin-resistant; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and dominant lethal effect of quinonolones on gyrase)

IT Gene, microbial  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (gyrA; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A)

IT Structure-activity relationship  
 (intein protein splicing-inhibiting; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

IT 82419-36-1, Ofloxacin  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (gyrase inhibitor; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and dominant lethal effect of quinonolones on gyrase)

IT 56-41-7, L-Alanine, biological studies  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (residue 172 of intein; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

IT 56-45-1, L-Serine, biological studies  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (residue 53 of intein; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

IT 56-86-0, L-Glutamic acid, biological studies  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (residue 65 of intein; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

IT 73-32-5, L-Isoleucine, biological studies  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (residues 21 and 97 of intein; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins)

in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

IT 72-19-5, L-Threonine, biological studies

RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(residues 3, 72, and 184 of intein; development of a pos. genetic selection system for inhibition of protein splicing using mycobacterial inteins in Escherichia coli DNA gyrase subunit A and isolation of intein mutations)

RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

- (1) Aspöck, G; Genome Res 1999, V9, P909 CAPLUS
- (2) Dalgaard, J; J Comput Biol 1997, V4, P193 CAPLUS
- (3) Daugelat, S; Protein Sci 1999, V8, P644 CAPLUS
- (4) Davis, E; EMBO J 1994, V13, P699 CAPLUS
- (5) Derbyshire, V; Proc Natl Acad Sci USA 1997, V94, P11466 CAPLUS
- (6) Duan, X; Cell 1997, V89, P555 CAPLUS
- (7) Fsihi, H; Proc Natl Acad Sci USA 1996, V93, P3410 CAPLUS
- (8) Gimble, F; FEMS Microbiol Lett 2000, V185, P99 CAPLUS
- (9) Guex, N; Electrophoresis 1997, V18, P2714 CAPLUS
- (10) Hall, T; Cell 1997, V91, P85 CAPLUS
- (11) Ichiiyanagi, K; J Mol Biol 2000, V300, P889 CAPLUS
- (12) Jacobs, M; Drugs 1999, V58, P19 CAPLUS
- (13) Jurica, M; Cell Mol Life Sci 1999, V55, P1304 CAPLUS
- (14) Kawasaki, M; J Biol Chem 1997, V272, P15668 CAPLUS
- (15) Klabunde, T; Nat Struct Biol 1998, V5, P31 CAPLUS
- (16) Lamden, K; Commun Dis Rep CDR Rev 1996, V6, P147 MEDLINE
- (17) Misteli, T; Nat Biotechnol 1997, V15, P961 CAPLUS
- (18) Nogami, S; Genetics 1997, V147, P73 CAPLUS
- (19) Noren, C; Angew Chem Int Ed Engl 2000, V39, P450 CAPLUS
- (20) Perler, F; Nucleic Acids Res 2000, V28, P344 CAPLUS
- (21) Perler, F; Trends in Biol Sci 1999, V24, P209 CAPLUS
- (22) Persidis, A; Nat Biotechnol 1998, V16, P100 CAPLUS
- (23) Pietrokovski, S; Protein Sci 1998, V7, P64 CAPLUS
- (24) Pietrokovski, S; Trends Genet 2001, V17, P465 CAPLUS
- (25) Poland, B; J Biol Chem 2000, V275, P16408 CAPLUS
- (26) Sander, P; Microbiology 1998, V144, P589 CAPLUS
- (27) Southworth, M; Embo J 1998, V17, P918 CAPLUS
- (28) Telenti, A; J Bacteriol 1997, V179, P6378 CAPLUS
- (29) Wood, D; Nat Biotechnol 1999, V17, P889 CAPLUS
- (30) Zaccolo, M; J Mol Biol 1996, V255, P589 CAPLUS

L6 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

AN 2001:779210 CAPLUS

DN 136:304984

ED Entered STN: 26 Oct 2001

TI DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells

AU Nakano, Masakazu; Ishimura, Masakazu; Chiba, Joe; Kanegae, Yumi; Saito, Izumu

CS Laboratory of Molecular Genetics, Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan

SO Microbiology and Immunology (2001), 45(9), 657-665  
CODEN: MIIMDV; ISSN: 0385-5600

PB Center for Academic Publications Japan

DT Journal

LA English

CC 3-4 (Biochemical Genetics)

Section cross-reference(s): 7

AB The FLP recombinase derived from *Saccharomyces cerevisiae* mediates precise site-specific recombination between a pair of FLP recognition targets (FRTs). Like the Cre/loxP system derived from bacteriophage P1, the FLP/FRT system has recently been applied to gene regulation systems using

an FLP-expressing recombinant adenovirus (rAd). In an attempt to improve the FLP/FRT system by altering its DNA substrates, we compared the recombination efficiency among different substrates by a quant. in vitro assay using FLP expressed in mammalian cells. Unexpectedly, we found that one linearized DNA substrate showed 4- to > 20-fold lower recombination efficiency than other substrates, which phenomenon has not been observed in the Cre/loxP system. The quant. in vitro assay using truncated DNA substrates suggested that the recombination efficiency seemed to be influenced not only by the linearized position of the substrate, but also by the length between a pair of FRTs. Such substrate preference of FLP expressed in mammalian cells should probably be noted when designing versatile applications of the FLP/FRT system as a gene regulation system in mammalian systems. Fortunately, however, we demonstrated that no substrate preference was observed when using a particular substrate (pCAFNF5) and the preference was reduced when using a certain pair of mutant FRTs (172), which will also be a promising tool for simultaneous gene regulation in combination with wild-type FRT.

- ST Saccharomyces FLP recognition target sequence recombinase; DNA substrate genetic recombination FLP recombinase
- IT Saccharomyces cerevisiae
  - (DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)
- IT Enzymes, biological studies
  - RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
  - (DNA-recombining, gene FLP; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)
- IT Genetic element
  - RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
  - (FRT (FLP recognition target); DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)
- IT DNA sequences
  - (FRT; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)
- IT Reporter gene
  - RL: BSU (Biological study, unclassified); BIOL (Biological study)
  - (GFP and LacZ; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)
- IT Structure-activity relationship
  - (enzyme substrate; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)
- IT Animal cell
  - (mammalian; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)
- IT Transgene
  - RL: BSU (Biological study, unclassified); BIOL (Biological study)
  - (pCAFNF5 with GFP and LacZ; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)
- IT Plasmids
  - (pCAFNF5; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)
- IT Recombination, genetic
  - (site-specific; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)
- IT 410802-79-8
  - RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
  - (nucleotide sequence; DNA substrates influence the recombination efficiency mediated by FLP recombinase expressed in mammalian cells)

RE.CNT 23      THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD  
RE

- (1) Andrews, B; Cell 1985, V40, P795 CAPLUS
- (2) Anton, M; J Virol 1995, V69, P4600 CAPLUS
- (3) Chen, Y; Mol Cell 2000, V6, P885 CAPLUS
- (4) Gopaul, D; Curr Opin Struct Biol 1999, V9, P14 CAPLUS
- (5) Guo, F; Nature 1997, V389, P40 CAPLUS
- (6) Guo, F; Proc Natl Acad Sci U S A 1999, V96, P7143 CAPLUS
- (7) Jayaram, M; Science 1997, V276, P49 CAPLUS
- (8) Kanegae, Y; Gene 1996, V181, P207 CAPLUS
- (9) Kanegae, Y; Nucleic Acids Res 1995, V23, P3816 CAPLUS
- (10) Kilby, N; Trends Genet 1993, V9, P413 CAPLUS
- (11) Lee, G; Gene 1998, V216, P55 CAPLUS
- (12) McLeod, M; Mol Cell Biol 1986, V6, P3357 CAPLUS
- (13) Miyake, S; Proc Natl Acad Sci U S A 1996, V93, P1320 CAPLUS
- (14) Nakano, M; Nucleic Acids Res 2001, V29, P40 MEDLINE
- (15) Ng, P; Mol Ther 2001, V3, P809 MEDLINE
- (16) Niwa, H; Gene 1991, V108, P193 CAPLUS
- (17) Sauer, B; Curr Opin Biotechnol 1994, V5, P521 CAPLUS
- (18) Schlake, T; Biochemistry 1994, V33, P12746 CAPLUS
- (19) Seibler, J; Biochemistry 1997, V36, P1740 CAPLUS
- (20) Senecoff, J; J Biol Chem 1986, V261, P7380 CAPLUS
- (21) Senecoff, J; Proc Natl Acad Sci U S A 1985, V82, P7270 CAPLUS
- (22) Umana, P; Nat Biotechnol 2001, V19, P582 MEDLINE
- (23) Umlauf, S; EMBO J 1988, V7, P1845 CAPLUS

L6    ANSWER 9 OF 11    BIOSIS    COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
DUPLICATE 2  
AN    2001:538663    BIOSIS  
DN    PREV200100538663  
TI    Green fluorescent protein rendered susceptible to proteolysis: Positions  
for protease-sensitive insertions.  
AU    Chiang, Cheng-Feng; Okou, David T.; Griffin, Tony B.; Verret, C. Reynold;  
Williams, Myron N. V. [Reprint author]  
CS    Department of Chemistry, Clark Atlanta University, Atlanta, GA, 30314, USA  
mnwill@cau.edu  
SO    Archives of Biochemistry and Biophysics, (October 15, 2001) Vol. 394, No.  
2, pp. 229-235. print.  
CODEN: ABBIA4. ISSN: 0003-9861.  
DT    Article  
LA    English  
ED    Entered STN: 21 Nov 2001  
Last Updated on STN: 25 Feb 2002  
AB    The green fluorescent protein (**GFP**) is highly resistant to  
proteolysis and remains uncleaved after prolonged incubation with trypsin  
or pronase despite several putative tryptic and chymotryptic sites in  
exposed loops. We have rendered **GFP** sensitive to proteolysis by  
inserting five amino acids, IEGRS, in loops at position 157, **172**  
, or 189. Excitation and emission maxima of the three insertion mutants  
were similar to those of wild type, but quantum yields of mutants OMEGA172  
and OMEGA189 were lower, indicating increased freedom of the fluorophore.  
Trypsin cleaved the native (folded) form of each mutant at a unique site  
defined by the insert. Pronase also yields similar digestion patterns in  
these variants, but further proteolysis was also observed, suggesting that  
the primary cleavage relaxes **GFP** structure and reveals  
previously inaccessible sites. Fluorescence of OMEGA189 changed little  
upon digestion with trypsin but decreased progressively by as much as 40%  
upon digestion with increasing amounts of pronase. Fluorescence of other  
variants was not affected significantly by the proteases, further  
confirming the remarkable stabilities of **GFP** variants. These  
constructs define a new conformation-sensitive site around residue 189 of  
**GFP** and show that **GFP** may be useful for design of



protease-susceptible molecules for monitoring of specific proteolytic activities in vivo.

CC Biochemistry studies - General 10060  
 Biochemistry studies - Proteins, peptides and amino acids 10064  
 Enzymes - General and comparative studies: coenzymes 10802  
 Physiology and biochemistry of bacteria 31000

IT Major Concepts  
 Biochemistry and Molecular Biophysics

IT Chemicals & Biochemicals  
 factor Xa; green fluorescent protein; pronase; protease; trypsin

IT Methods & Equipment  
 mutagenesis: analytical method, genetic engineering, genetic method

IT Miscellaneous Descriptors  
 protease-sensitive insertions; proteolysis

ORGN Classifier  
 Bacteria 05000  
 Super Taxa  
 Microorganisms  
 Organism Name  
 bacteria  
 Taxa Notes  
 Bacteria, Eubacteria, Microorganisms

RN 9002-05-5 (factor Xa)  
 9036-06-0 (pronase)  
 9001-92-7 (protease)  
 9002-07-7 (trypsin)

L6 ANSWER 10 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
 DUPLICATE 3

AN 2000:129563 BIOSIS

DN PREV200000129563

TI Post-translational regulation of Adr1 activity is mediated by its DNA  
 binding domain.

AU Sloan, James S.; Dombeck, Kenneth M.; Young, Elton T. [Reprint author]

CS Department of Biochemistry, University of Washington, Seattle, WA,  
 98195-7350, USA

SO Journal of Biological Chemistry, (Dec. 31, 1999) Vol. 274, No. 53, pp.  
 37575-37582. print.  
 CODEN: JBCHA3. ISSN: 0021-9258.

DT Article

LA English

ED Entered STN: 12 Apr 2000  
 Last Updated on STN: 4 Jan 2002

AB ADL1 encodes a transcriptional activator that regulates genes involved in  
 carbon source utilization in *Saccharomyces cerevisiae*. ADL1 is itself  
 repressed by glucose, but the significance of this repression for  
 regulating target genes is not known. To test if the reduction in Adl1  
 levels contributes to glucose repression of ADH2 expression, we generated  
 yeast strains in which the level of Adl1 produced during growth in  
 glucose-containing medium is similar to that present in wild-type cells  
 grown in the absence of glucose. In these Adl1-overproducing strains,  
 ADH2 expression remained tightly repressed, and UAS1, the element in the  
 ADH2 promoter that binds Adl1, was sufficient to maintain glucose  
 repression. Post-translational modification of Adl1 activity is  
 implicated in repression, since ADH2 derepression occurred in the absence  
 of de novo protein synthesis. The N-terminal 172 amino acids of  
 Adl1, containing the DNA binding and nuclear localization domains, fused  
 to the Herpesvirus VP16-encoded transcription activation domain, conferred  
 regulated expression at UAS1. Nuclear localization of an Adl1-GFP  
 fusion protein was not glucose-regulated, suggesting that the DNA binding  
 domain of Adl1 is sufficient to confer regulated expression on target  
 genes. A Gal4-Adl1 fusion protein was unable to confer glucose repression

at GAL4-dependent promoters, suggesting that regulation mediated by ADRI1 is specific to UAS1.

CC Genetics - Plant 03504  
 Biochemistry studies - General 10060

IT Major Concepts  
 Molecular Genetics (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals  
 Adr1 protein: DNA binding domain, activity, post-translational regulation; *Saccharomyces cerevisiae* ADH2 gene

L6 ANSWER 11 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
 DUPLICATE 4

AN 1998:509211 BIOSIS  
 DN PREV199800509211

TI UGA codon position affects the efficiency of selenocysteine incorporation into glutathione peroxidase-1.

AU Wen, Wu; Weiss, Sherri L.; Sunde, Roger A. [Reprint author]  
 CS 217 Gwynn Hall, Univ. Missouri, Columbia, MO 65211, USA  
 SO Journal of Biological Chemistry, (Oct. 23, 1998) Vol. 273, No. 43, pp. 28533-28541. print.  
 CODEN: JBCHA3. ISSN: 0021-9258.

DT Article  
 LA English  
 ED Entered STN: 18 Dec 1998  
 Last Updated on STN: 10 May 1999

AB AUGA codon and a selenocysteine insertion sequence in the 3'-untranslated region are the only established mRNA elements necessary for selenocysteine (Sec or U) incorporation during translation. These two elements, however, do not universally confer efficient Sec incorporation. The objective of this study was to systematically examine the effect of UGA codon position on efficiency of Sec insertion. In a glutathione peroxidase-1 (F-GPX1) expression vector, the UGA at the native position (U47) was mutated to a cysteine codon, and codons for Ser-7, Ser-12, Ser-18, Ser-29, Ser-45, Ser-93, Cys-154, Val-172, Ser-178, and Ser-195 were individually mutated to UGA and transiently expressed in COS-7 cells. 75Se incorporation at the 11 positions was 31, 72, 54, 105, 90, 100, 146, 135, 13, 11, and 43%, respectively, of 75Se incorporation at U47, suggesting that Sec is more efficiently incorporated at UGA codons positioned in the middle of the coding region rather than close to the 5' or 3' ends. Ribonuclease protection showed that these differences were not due to differences in mRNA level. When the green fluorescence protein (GFP) coding region was placed in-frame at the 5' or 3' ends of the coding region in F-GPX1 to produce chimeric 50-51-kDa GFP/GPX1 proteins, Sec incorporation at UGA codons, formerly close to the 5' or 3' ends, was increased to levels comparable to the UGA at U47. Insertion of GFP after the UAA-stop was just as effective in increasing Sec insertion efficiency as GFP inserted before the stop. These studies used a recombinant expression model that incorporated Sec at non-native UGA codons at rates equal to those of endogenous glutathione peroxidase-1 and showed that the efficiency of Sec incorporation can be modulated by UGA position; Sec incorporation at high efficiency appears to require that the UGA be >21 nucleotides from the AUG-start and >204 nucleotides from the selenocysteine insertion sequence element.

CC Enzymes - General and comparative studies: coenzymes 10802  
 Cytology - Animal 02506  
 Biochemistry methods - General 10050  
 Biochemistry studies - General 10060

IT Major Concepts  
 Enzymology (Biochemistry and Molecular Biophysics); Methods and Techniques

IT Chemicals & Biochemicals  
 glutathione peroxidase-1: analysis; selenocysteine: analysis,

ANSWER 1 OF 1 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

DUPLICATE 1

AN 1997:344876 BIOSIS

DN PREV199799644079

TI An improved GFP cloning cassette designed for prokaryotic transcriptional fusions.

AU Miller, William G.; Lindow, Steven E. [Reprint author]

CS Dep. Plant Microbial Biol., Univ. Calif., 111 Koshland Hall, Berkeley, CA 94720, USA

SO Gene (Amsterdam), (1997) Vol. 191, No. 2, pp. 149-153.

CODEN: GENED6. ISSN: 0378-1119.

DT Article

LA English

ED Entered STN: 11 Aug 1997

Last Updated on STN: 11 Aug 1997

AB A new gfp cloning cassette designed for prokaryotic transcriptional fusions has been constructed. This cassette consists of gfp (containing the S65T 'red-shift' (Heim et al. (1995) Nature 373, 663-6641 and F64L 'protein solubility' (Cormack et al. (1996) Gene 173, 33-38) mutations) flanked by convenient restriction sites, a translational enhancer, and a consensus ribosome binding site with an optimized spacer region. gfp fusion strains containing this cassette demonstrate from 40- to 80-fold greater fluorescence intensity than **wild-type gfp** fusion strains. Additionally, this cassette confers sufficient fluorescence to recipient cells to be used in low copy-number plasmids, with promoters conferring low levels of transcription, and in bacterial taxa other than Escherichia, such as Pseudomonas.

CC Genetics - General 03502

Biochemistry studies - Nucleic acids, purines and pyrimidines 10062

Replication, transcription, translation 10300

Physiology and biochemistry of bacteria 31000

Genetics of bacteria and viruses 31500

IT Major Concepts

Biochemistry and Molecular Biophysics; Genetics; Molecular Genetics (Biochemistry and Molecular Biophysics); Physiology

IT Miscellaneous Descriptors

CONSENSUS RIBOSOME BINDING SITE; CONSTRUCTION; DESIGN; GENETIC TOOL; GREEN FLUORESCENT PROTEIN CLONING CASSETTE; GREEN FLUORESCENT PROTEIN GENE; MOLECULAR GENETICS; OPTIMIZER SPACER REGION; PGREENTIR; PLASMID; PROMOTER; REPORTER GENE; RESTRICTION SITES; TRANSCRIPTIONAL FUSION; TRANSCRIPTIONAL REGULATION; TRANSLATIONAL ENHANCER

ORGN Classifier

Enterobacteriaceae 06702

Super Taxa

Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms

Organism Name

Escherichia coli

Taxa Notes

Bacteria, Eubacteria, Microorganisms

ORGN Classifier

Pseudomonadaceae 06508

Super Taxa

Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms

Organism Name

Pseudomonas syringae

Taxa Notes

Bacteria, Eubacteria, Microorganisms

=>

June 1997

DUPLICATE 1

AN 1997:344876 BIOSIS

DN PREV199799644079

TI An improved GFP cloning cassette designed for prokaryotic transcriptional fusions.

AU Miller, William G.; Lindow, Steven E. [Reprint author]

CS Dep. Plant Microbial Biol., Univ. Calif., 111 Koshland Hall, Berkeley, CA 94720, USA

SO Gene (Amsterdam), (1997) Vol. 191, No. 2, pp. 149-153.

CODEN: GENED6. ISSN: 0378-1119.

DT Article

LA English

ED Entered STN: 11 Aug 1997

Last Updated on STN: 11 Aug 1997

AB A new gfp cloning cassette designed for prokaryotic transcriptional fusions has been constructed. This cassette consists of gfp (containing the S65T 'red-shift' (Heim et al. (1995) Nature 373, 663-6641 and F64L 'protein solubility' (Cormack et al. (1996) Gene 173, 33-38) mutations) flanked by convenient restriction sites, a translational enhancer, and a consensus ribosome binding site with an optimized spacer region. gfp fusion strains containing this cassette demonstrate from 40- to 80-fold greater fluorescence intensity than **wild-type gfp** fusion strains. Additionally, this cassette confers sufficient fluorescence to recipient cells to be used in low copy-number plasmids, with promoters conferring low levels of transcription, and in bacterial taxa other than Escherichia, such as Pseudomonas.

CC Genetics - General 03502

Biochemistry studies - Nucleic acids, purines and pyrimidines 10062

Replication, transcription, translation 10300

Physiology and biochemistry of bacteria 31000

Genetics of bacteria and viruses 31500

IT Major Concepts

Biochemistry and Molecular Biophysics; Genetics; Molecular Genetics (Biochemistry and Molecular Biophysics); Physiology

IT Miscellaneous Descriptors

CONSENSUS RIBOSOME BINDING SITE; CONSTRUCTION; DESIGN; GENETIC TOOL; GREEN FLUORESCENT PROTEIN CLONING CASSETTE; GREEN FLUORESCENT PROTEIN GENE; MOLECULAR GENETICS; OPTIMIZER SPACER REGION; PGREENTIR; PLASMID; PROMOTER; REPORTER GENE; RESTRICTION SITES; TRANSCRIPTIONAL FUSION; TRANSCRIPTIONAL REGULATION; TRANSLATIONAL ENHANCER

ORGN Classifier

Enterobacteriaceae 06702

Super Taxa

Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms

Organism Name

Escherichia coli

Taxa Notes

Bacteria, Eubacteria, Microorganisms

ORGN Classifier

Pseudomonadaceae 06508

Super Taxa

Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms

Organism Name

Pseudomonas syringae

Taxa Notes

Bacteria, Eubacteria, Microorganisms

d his

(FILE 'HOME' ENTERED AT 14:57:26 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT  
14:58:00 ON 26 APR 2004

L1	301 S (WILD TYPE GFP)
L2	0 S L1 AND 157
L3	0 S L1 AND 157
L4	0 S L1 AND 158
L5	0 S L1 AND 172
L6	3 S L1 AND 173
L7	1 DUPLICATE REMOVE L6 (2 DUPLICATES REMOVED)

=>

d his

(FILE 'HOME' ENTERED AT 14:57:26 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT  
14:58:00 ON 26 APR 2004

L1	301 S (WILD TYPE GFP)
L2	0 S L1 AND 157
L3	0 S L1 AND 157
L4	0 S L1 AND 158
L5	0 S L1 AND 172
L6	3 S L1 AND 173
L7	1 DUPLICATE REMOVE L6 (2 DUPLICATES REMOVED)

=>

ANSWER 1 OF 2 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

AN 1997:174907 BIOSIS  
 DN PREV199799466620  
 TI A novel mutation which enhances the fluorescence of green fluorescent **protein** at high temperatures.  
 AU Kimata, Yukio; Iwaki, Masaharu; Lim, Chun Ren; Kohno, Kenji [Reprint author]  
 CS Res. Education Cent. Genetic Information, Nara Inst. Sci. Technol., 8916-5, Takayama, Ikoma, Nara 630-01, Japan  
 SO Biochemical and Biophysical Research Communications (1997) Vol. 232, No. 1, pp. 69-73. *check*  
 CODEN: BBRCA9. ISSN: 0006-291X.  
 DT Article  
 LA English  
 ED Entered STN: 24 Apr 1997  
 Last Updated on STN: 24 Apr 1997

AB Green fluorescent **protein** (GFP) from *Aequorea victoria* is widely used as a marker of gene expression and **protein** localization in living cells from **prokaryotes** to eukaryotes. However, the total fluorescent signal from **wild-type GFP** is very weak when expressed in cells cultured at 37 degree C compared to 30 degree C or below. This characteristic makes GFP poorly suited to use as a marker in mammalian cells. Here we describe a new variant of GFP which carries a substitution of **Ser-147** to **Pro** (S147P GFP) and which emits a stronger fluorescent signal than the **wild-type GFP** at high temperature. When S147P is combined with the **Ser-65** to **Thr** mutation (S65T GFP), the resulting double mutant emits fluorescence which is several-fold stronger than GFP with a single S65T modification in both bacterial or mammalian cells. This S147P mutation should be useful for constructing new GFP variants which stably emit strong fluorescence at a wide range of culturing temperatures.

\* CC Genetics - Animal 03506  
 Biochemistry studies - General 10060  
 Biophysics - General 10502  
 Temperature - General measurement and methods 23001  
 IT Major Concepts  
 Biochemistry and Molecular Biophysics; Genetics; Methods and Techniques  
 IT Miscellaneous Descriptors  
 FLUORESCENCE; GREEN FLUORESCENT **PROTEIN**; HIGH TEMPERATURES;  
 MOLECULAR GENETICS; MUTANT; MUTATION; WILD-TYPE  
 ORGN Classifier  
 Cnidaria 41000  
 Super Taxa  
 Invertebrata; Animalia  
 Organism Name  
*Aequorea victoria*  
 Taxa Notes  
 Animals, Invertebrates

L4 ANSWER 2 OF 2 JAPIO (C) 2004 JPO on STN  
 AN 1998-234382 JAPIO  
 TI FLUORESCENT **PROTEIN**  
 IN KONO KENJI; TAKEDA KATSUO; HASEGAWA MAMORU  
 PA DEINABETSUKU KENKYUSHO:KK  
 PI JP 10234382 A 19980908 Heisei  
 AI JP 1997-62370 (JP09062370 Heisei) 19970227  
 PRAI JP 1997-62370 19970227  
 SO PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 1998  
 IC ICM C12N015-09  
 ICS C07H021-04; C07K014-435; C12N001-21; C12N005-10; C12P021-02;  
 C12Q001-68

March 1997

ANSWER 1 OF 2 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

AN 1997:174907 BIOSIS  
DN PREV199799466620  
TI A novel mutation which enhances the fluorescence of green fluorescent **protein** at high temperatures.  
AU Kimata, Yukio; Iwaki, Masaharu; Lim, Chun Ren; Kohno, Kenji [Reprint author]  
CS Res. Education Cent. Genetic Information, Nara Inst. Sci. Technol., 8916-5, Takayama, Ikoma, Nara 630-01, Japan  
SO Biochemical and Biophysical Research Communications, (1997) Vol. 232, No. 1, pp. 69-73.  
CODEN: BBRCA9. ISSN: 0006-291X.  
DT Article  
LA English  
ED Entered STN: 24 Apr 1997  
Last Updated on STN: 24 Apr 1997  
AB Green fluorescent **protein** (GFP) from *Aequorea victoria* is widely used as a marker of gene expression and **protein** localization in living cells from **prokaryotes** to eukaryotes. However, the total fluorescent signal from **wild-type GFP** is very weak when expressed in cells cultured at 37 degree C compared to 30 degree C or below. This characteristic makes GFP poorly suited to use as a marker in mammalian cells. Here we describe a new variant of GFP which carries a substitution of **Ser-147** to **Pro** (S147P GFP) and which emits a stronger fluorescent signal than the **wild-type GFP** at high temperature. When S147P is combined with the **Ser-65** to Thr mutation (S65T GFP), the resulting double mutant emits fluorescence which is several-fold stronger than GFP with a single S65T modification in both bacterial or mammalian cells. This S147P mutation should be useful for constructing new GFP variants which stably emit strong fluorescence at a wide range of culturing temperatures.  
CC Genetics - Animal 03506  
Biochemistry studies - General 10060  
Biophysics - General 10502  
Temperature - General measurement and methods 23001  
IT Major Concepts  
Biochemistry and Molecular Biophysics; Genetics; Methods and Techniques  
IT Miscellaneous Descriptors  
FLUORESCENCE; GREEN FLUORESCENT **PROTEIN**; HIGH TEMPERATURES;  
MOLECULAR GENETICS; MUTANT; MUTATION; WILD-TYPE  
ORGN Classifier  
Cnidaria 41000  
Super Taxa  
Invertebrata; Animalia  
Organism Name  
*Aequorea victoria*  
Taxa Notes  
Animals, Invertebrates

L4 ANSWER 2 OF 2 JAPIO (C) 2004 JPO on STN  
AN 1998-234382 JAPIO  
TI FLUORESCENT **PROTEIN**  
IN KONO KENJI; TAKEDA KATSUO; HASEGAWA MAMORU  
PA DEINABETSUKU KENKYUSHO:KK  
PI JP 10234382 A 19980908 Heisei  
AI JP 1997-62370 (JP09062370 Heisei) 19970227  
PRAI JP 1997-62370 19970227  
SO PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 1998  
IC ICM C12N015-09  
ICS C07H021-04; C07K014-435; C12N001-21; C12N005-10; C12P021-02;  
C12Q001-68



ICI C12N001-21, C12R001:19; C12N005-10, C12R001:91; C12P021-02, C12R001:19  
AB **PROBLEM** TO BE SOLVED: To obtain a fluorescent **protein** capable of being expressed even by the culture of a host cell at a high temperature (37&deg;C), emitting stronger fluorescent light than those of conventional fluorescent **proteins** (GFP), and useful as a labeling agent for the analyses of **protein** localization in live cells, a reporter for the analyses of **promoters**, etc., by introducing two mutation amino acids into a **wild type GFP**.

SOLUTION: This fluorescent **protein** is obtained by mutating the Number 147 **serine** and the Number 65 **serine** of the cDNA of a **wild type GFP** with **proline** and threonine, respectively, by a site-specific mutation method, etc., transforming Escherichia coil with a plasmid containing the obtained GFPcDNA and subsequently expressing the mutated GFP containing an amino acid sequence of the formula in the Escherichia coil at a high temperature (37&deg;C). The fluorescent **protein** emits about three-fold fluorescent light that of S65T mutant, is contained in a higher concentration than that of the S65T mutant, when expressed in the cell, and emits the fluorescent light under a high temperature (37&deg;C).  
COPYRIGHT: (C)1998,JPO

=>

ICI C12N001-21, C12R001:19; C12N005-10, C12R001:91; C12P021-02, C12R001:19  
AB **PROBLEM** TO BE SOLVED: To obtain a fluorescent **protein** capable of being expressed even by the culture of a host cell at a high temperature (37&deg;C), emitting stronger fluorescent light than those of conventional fluorescent **proteins** (GFP), and useful as a labeling agent for the analyses of **protein** localization in live cells, a reporter for the analyses of **promoters**, etc., by introducing two mutation amino acids into a **wild type GFP**.

SOLUTION: This fluorescent **protein** is obtained by mutating the Number 147 **serine** and the Number 65 **serine** of the cDNA of a **wild type GFP** with **proline** and threonine, respectively, by a site-specific mutation method, etc., transforming Escherichia coil with a plasmid containing the obtained GFPcDNA and subsequently expressing the mutated GFP containing an amino acid sequence of the formula in the Escherichia coil at a high temperature (37&deg;C). The fluorescent **protein** emits about three-fold fluorescent light that of S65T mutant, is contained in a higher concentration than that of the S65T mutant, when expressed in the cell, and emits the fluorescent light under a high temperature (37&deg;C).  
COPYRIGHT: (C)1998,JPO

=>

DUPLICATE 1

AN 2001:538663 BIOSIS

DN PREV200100538663

TI Green fluorescent protein rendered susceptible to proteolysis: Positions for protease-sensitive insertions.

AU Chiang, Cheng-Feng; Okou, David T.; Griffin, Tony B.; Verret, C. Reynold; Williams, Myron N. V. [Reprint author]

CS Department of Chemistry, Clark Atlanta University, Atlanta, GA, 30314, USA  
mnwill@cau.edu

SO Archives of Biochemistry and Biophysics, (October 15, 2001) Vol. 394, No. 2, pp. 229-235. print.

CODEN: ABBIA4. ISSN: 0003-9861.

DT Article

LA English

ED Entered STN: 21 Nov 2001

Last Updated on STN: 25 Feb 2002

AB The green fluorescent protein (**GFP**) is highly resistant to proteolysis and remains uncleaved after prolonged incubation with trypsin or pronase despite several putative tryptic and chymotryptic sites in exposed loops. We have rendered **GFP** sensitive to proteolysis by inserting five amino acids, IEGRS, in loops at position **157**, **172**, or 189. Excitation and emission maxima of the three insertion mutants were similar to those of wild type, but quantum yields of mutants OMEGA172 and OMEGA189 were lower, indicating increased freedom of the fluorophore. Trypsin cleaved the native (folded) form of each mutant at a unique site defined by the insert. Pronase also yields similar digestion patterns in these variants, but further proteolysis was also observed, suggesting that the primary cleavage relaxes **GFP** structure and reveals previously inaccessible sites. Fluorescence of OMEGA189 changed little upon digestion with trypsin but decreased progressively by as much as 40% upon digestion with increasing amounts of pronase. Fluorescence of other variants was not affected significantly by the proteases, further confirming the remarkable stabilities of **GFP** variants. These constructs define a new conformation-sensitive site around residue 189 of **GFP** and show that **GFP** may be useful for design of protease-susceptible molecules for monitoring of specific proteolytic activities in vivo.

CC Biochemistry studies - General 10060

Biochemistry studies - Proteins, peptides and amino acids 10064

Enzymes - General and comparative studies: coenzymes 10802

Physiology and biochemistry of bacteria 31000

IT Major Concepts

Biochemistry and Molecular Biophysics

IT Chemicals &amp; Biochemicals

factor Xa; green fluorescent protein; pronase; protease; trypsin

IT Methods &amp; Equipment

mutagenesis: analytical method, genetic engineering, genetic method

IT Miscellaneous Descriptors

protease-sensitive insertions; proteolysis

ORGN Classifier

Bacteria 05000

Super Taxa

Microorganisms

Organism Name

bacteria

Taxa Notes

Bacteria, Eubacteria, Microorganisms

RN 9002-05-5 (factor Xa)

9036-06-0 (pronase)

9001-92-7 (protease)

9002-07-7 (trypsin)

DUPLICATE 1

AN 2001:538663 BIOSIS

DN PREV200100538663

TI Green fluorescent protein rendered susceptible to proteolysis: Positions for protease-sensitive insertions.

AU Chiang, Cheng-Feng; Okou, David T.; Griffin, Tony B.; Verret, C. Reynold; Williams, Myron N. V. [Reprint author]

CS Department of Chemistry, Clark Atlanta University, Atlanta, GA, 30314, USA  
mnwill@cau.edu

SO Archives of Biochemistry and Biophysics, (October 15, 2001) Vol. 394, No. 2, pp. 229-235. print.

CODEN: ABBIA4. ISSN: 0003-9861.

DT Article

LA English

ED Entered STN: 21 Nov 2001

Last Updated on STN: 25 Feb 2002

AB The green fluorescent protein (**GFP**) is highly resistant to proteolysis and remains uncleaved after prolonged incubation with trypsin or pronase despite several putative tryptic and chymotryptic sites in exposed loops. We have rendered **GFP** sensitive to proteolysis by inserting five amino acids, IEGRS, in loops at position 157, 172, or 189. Excitation and emission maxima of the three insertion mutants were similar to those of wild type, but quantum yields of mutants OMEGA172 and OMEGA189 were lower, indicating increased freedom of the fluorophore. Trypsin cleaved the native (folded) form of each mutant at a unique site defined by the insert. Pronase also yields similar digestion patterns in these variants, but further proteolysis was also observed, suggesting that the primary cleavage relaxes **GFP** structure and reveals previously inaccessible sites. Fluorescence of OMEGA189 changed little upon digestion with trypsin but decreased progressively by as much as 40% upon digestion with increasing amounts of pronase. Fluorescence of other variants was not affected significantly by the proteases, further confirming the remarkable stabilities of **GFP** variants. These constructs define a new conformation-sensitive site around residue 189 of **GFP** and show that **GFP** may be useful for design of protease-susceptible molecules for monitoring of specific proteolytic activities in vivo.

CC Biochemistry studies - General 10060

Biochemistry studies - Proteins, peptides and amino acids 10064

Enzymes - General and comparative studies: coenzymes 10802

Physiology and biochemistry of bacteria 31000

IT Major Concepts

Biochemistry and Molecular Biophysics

IT Chemicals &amp; Biochemicals

factor Xa; green fluorescent protein; pronase; protease; trypsin

IT Methods &amp; Equipment

mutagenesis: analytical method, genetic engineering, genetic method

IT Miscellaneous Descriptors

protease-sensitive insertions; proteolysis

ORGN Classifier

Bacteria 05000

Super Taxa

Microorganisms

Organism Name

bacteria

Taxa Notes

Bacteria, Eubacteria, Microorganisms

RN 9002-05-5 (factor Xa)

9036-06-0 (pronase)

9001-92-7 (protease)

9002-07-7 (trypsin)

ANSWER 3 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

AN 2003:296076 CAPLUS

DN 138:315791

ED Entered STN: 17 Apr 2003

TI Fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or  $\beta$ -lactamase

IN Anderson, David; Peelle, Beau Robert; Bogenberger, Jakob Maria

PA Rigel Pharmaceuticals, Inc., USA

SO U.S., 63 pp., Cont.-in-part of U.S. 6,180,343.

CODEN: USXXAM

DT Patent

LA English

IC ICM C07K004-00

ICS C07K014-435; C12Q001-68; C12N015-63; C12N015-12

NCL 530300000; 530350000; 435006000; 435320100; 536023400; 536023500

CC 3-1 (Biochemical Genetics)

Section cross-reference(s): 9

FAN.CNT 4

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6548632	B1	20030415	US 1999-415765	19991008
	US 6180343	B1	20010130	US 1998-169015	19981008
	US 6548249	B1	20030415	US 2000-626581	20000727
	US 6562617	B1	20030513	US 2000-626580	20000727
	US 2001003650	A1	20010614	US 2000-749959	20001227
	US 6596485	B2	20030722		
	US 2003143562	A1	20030731	US 2002-177725	20020620
	US 2003224412	A1	20031204	US 2003-393449	20030318
PRAI	US 1998-169015	A2	19981008		
	US 1999-415765	A3	19991008		
	US 2002-177725	A2	20020620		

AB The invention relates to the use of scaffold proteins, particularly green fluorescent protein (**GFP**) and  $\beta$ -lactamase TEM-1, in fusion constructs with random and defined peptides and peptide libraries. The fusions act to increase the cellular expression levels, decrease the cellular catabolism, increase the conformational stability relative to linear peptides, and increase the steady state concns. of the random peptides and random peptide library members expressed in cells for the purpose of detecting the presence of the peptides and screening random peptide libraries. N-terminal, C-terminal, dual N- and C-terminal, and one or more internal fusions are all contemplated. Internal fusions in Renilla **GFP** may be made in loops 1-5 (amino acid residues 130-135, 154-159, 172-175, 188-193, or 208-216) for optimal presentation of the peptide. Inclusion of multiple highly flexible amino acid residues between **GFP** and the library allows minimal conformational constraints on the **GFP**. Designed insertion sites in loops 2-4 retain a high level of **GFP** fluorescence when the inserts are flanked by multiple glycines in the tetrapeptide linkers. Novel fusions utilizing self-binding peptides to create a conformationally stabilized fusion domain are also contemplated.

ST scaffold protein fusion random peptide library; green fluorescent protein fusion random peptide library; lactamase fusion random peptide library

IT Animal cell line

(293, **GFP** fusions expressed in; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or  $\beta$ -lactamase)

IT Aequorea

Renilla reniformis

(**GFP** from; fusions of random peptide libraries in scaffold proteins such as green fluorescent protein or  $\beta$ -lactamase)

IT Animal cell line

(JURKAT, **GFP** fusions expressed in; fusions of random peptide

libraries in scaffold proteins such as green fluorescent protein or  
β-lactamase)

IT Antigen presentation

Peptide library

(fusions of random peptide libraries in scaffold proteins such as green

d his

(FILE 'HOME' ENTERED AT 15:24:06 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT  
15:24:34 ON 26 APR 2004

L1	19 S GFP AND 157
L2	24 S GFP AND 172
L3	4 S L1 AND L2
L4	1 DUPLICATE REMOVE L3 (3 DUPLICATES REMOVED)
L5	6 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)
L6	11 DUPLICATE REMOVE L2 (13 DUPLICATES REMOVED)

=>

d his

(FILE 'HOME' ENTERED AT 15:24:06 ON 26 APR 2004)

FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT  
15:24:34 ON 26 APR 2004

L1	19 S GFP AND 157
L2	24 S GFP AND 172
L3	4 S L1 AND L2
L4	1 DUPLICATE REMOVE L3 (3 DUPLICATES REMOVED)
L5	6 DUPLICATE REMOVE L1 (13 DUPLICATES REMOVED)
L6	11 DUPLICATE REMOVE L2 (13 DUPLICATES REMOVED)

=>